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DEVELOPMENT OF A MOBILE OPEN-CIRCUIT SYSTEM BASED ON INDIRECT CALORIMETRY FOR ENERGETIC METABOLISM STUDIES IN SMALL RUMINANTS

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

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Abbreviations

A
ADF: acid detergent fiber

B
BW: body weight

C
CN: carbon-nitrogen balance
CO$_{2x}$: CO_2 production from oxidation
CP: crude protein
CS: comparative slaughter technique

D
DM: dry matter
DMI: dry matter intake

E
EE: ether extract
E$_{milk}$: energy retained in milk

F
FHP: fasting heat production

G
GE: gross energy
GEI: gross energy intake

H
HP: heat production
HPf: heat production from fermentation
HPx: heat production from oxidation

K
K$_g$: efficiency of use of ME for tissue gain
K$_l$: efficiency of use of ME for milk production
K$_m$: efficiency of use of ME for maintenance
K$_t$: efficiency of use of body stores for milk production

M
ME: metabolizable energy
MEA: metabolizable energy available for productive purposes

MEI: metabolizable energy intake
MEm: metabolizable energy for maintenance

N
NDF: neutral detergent fiber
NDSF: neutral detergent soluble fiber
NE$_L$: net energy for lactation
NEm: net energy for maintenance
NFC: non fibrous carbohydrates
N$_{urine}$: urine nitrogen

O
OM: organic matter
OXCHO: oxidation of carbohydrate
OXF: oxidation of fat
OXP: oxidation of protein

P
Prop: fraction of MEA used for milk synthesis
Prop$_{max}$: maximum fraction of MEA that can be used for milk production

R
RE: retained energy
RE$_{body}$: retained energy in body tissues
RE$_{fat}$: retained energy as fat
RE$_{protein}$: retained energy as protein
RQ: respiratory quotient
RQnpx: non protein respiratory quotient for oxidation of nutrients

S
SEM: standard error of the mean

V
VFA: volatile fatty acids

Z
Z: level of MEA at which 50% of maximum fraction of MEA is used for milk production

DIETS

Experiment II:
  CGR: diet whose concentrate is based on cereal grain (barley)
  FCH: diet whose concentrate is based on fibrous carbohydrates (soybean hulls and corn gluten feed)
  ALH: diet of alfalfa hay

Experiment III:
  BRL: diet whose concentrate is based on barley grain
  SHCG: diet whose concentrate is based on soybean hulls and corn gluten feed

Experiment IV:
  CRN: diet whose concentrate is based on corn grain
  SHGF: diet whose concentrate is based on soybean hulls and corn gluten feed

Experiment V:
  CRG: diet whose concentrate is based on corn grain
  CTP: diet whose concentrate is based on dry citrus pulp
  SYH: diet whose concentrate is based on soybean hulls

ABSTRACT - RESUMEN - RESUM

ABSTRACT

For many years energy needs of ruminants have tried to be known to formulate rations adjusted, but it has been found that there are a variety of factors that affect them. Therefore, lots of studies are needed for evaluating the effect of these factors.

Consequently, the main objective of this Thesis was to design and validate a respirometry system based on indirect calorimetry, which would allow assessing energy needs of small ruminants accurately. It was intended from the beginning it was a mobile system and of relatively low cost. Furthermore, a methane gas analyzer was incorporated to this system, which allowed the measurement of emissions of this greenhouse gas and quantification of energy losses in the form of methane.

Initially the system had connected a mask, which was placed on the animal's face. A sample of exhaled gas was stored in a gas collection bag which was connected to the analyzer, and it measured the concentration of O_2, CO_2 and CH_4 from the air. The proper functioning of the system was checked by a pilot experiment with dry Murciano-Granadina breed goats fed at maintenance level. Later this system was improved. Some of the most important changes were the replacement of the mask by a head hood in which the animal introduced the whole head, and the development of software that recorded and kept automatically concentrations of O_2, CO_2 and CH_4 in exhaled air. This improvement allowed gas measurements during longer periods of time and recording more data. These changes were also validated through a pilot test with dry Manchega breed sheep.

Subsequently, three experiments were performed. Diets were mixed rations that differed in the inclusion of cereal or fibrous by-products. The effect of diet was studied on digestibility, energy and carbon-nitrogen balances, nutrients oxidation, rumen parameters, methane production and, where appropriate, on milk performance. Dry Guirra ewes were used in the first experiment and it was found that replacement of cereal by fibrous by-products had no negative effect on energy metabolism and resulted in positive energy balance (62 kJ/kg $BW^{0.75}$, on average). In the other two experiments Murciano-Granadina goats during mid lactation were used. In the first one, the diet not affected the energy balance, which was positive, with an average value of metabolizable energy intake of 1,444 kJ/kg $BW^{0.75}$. In the next experiment goats showed negative energy balance, that was accentuated with the replacement of cereal by fibrous by-products. The most unfavorable case was the fibrous diet which presented a fat oxidation of 74.4% compared to carbohydrate oxidation of 18.5%. Methane production was also higher for fibrous diets (34.8 g/d, on average) than for cereal diet (24.7 g/d). In both studies with lactating goats no effect of the diet on milk yield was observed; however, the fat content of milk increased, like acetic acid production in the rumen.

The determination of the calibration factor for O_2 (1.005 ± 0.0101) confirmed the proper functioning of equipment. Moreover, small differences between the heat

production obtained by indirect calorimetry and the carbon-nitrogen balance (2% in sheep and 1% in goats) demonstrated that this system allows determining the heat production of the animals reliably and accurately.

Methane emission of dry and non-pregnant sheep was 18.2 g/d and it of lactating goats was 30.7 g/d. Moreover, in the experiments of this Thesis have been estimated maintenance energy needs of two Spanish native sheep breeds, such as the sheep from the Guirra and Manchega breeds, by lineal regression or measurement of fasting heat production, respectively; net maintenance requirements were 270 kJ/kg $BW^{0.75}$, on average. In the case of Murciano-Granadina breed goats, in the middle of lactation, the average utilization efficiency of metabolizable energy for lactation was 0.61.

RESUMEN

Desde hace años se ha tratado de conocer las necesidades energéticas de los rumiantes con el fin de formular raciones ajustadas, pero se ha comprobado que hay una gran variedad de factores que les afectan; por ello son necesarios estudios que evalúen el efecto de estos factores. Como consecuencia, el principal objetivo de esta tesis fue diseñar y validar un equipo de respirometría, basado en calorimetría indirecta, que permitiese evaluar las necesidades en energía de pequeños rumiantes de forma precisa. Se pretendió desde el inicio que fuese un sistema móvil y de relativo bajo coste. Además, a este sistema también se le incorporó un analizador de gas metano, que permitía la medición de las emisiones de este gas de efecto invernadero y la cuantificación de las pérdidas energéticas en forma de metano.

Inicialmente el equipo tenía conectada una máscara que se colocaba en la cara del animal. Una muestra del gas espirado era almacenada en una bolsa de recogida de gases que era conectada al analizador, el cual medía la concentración de O_2, CO_2 y CH_4 del aire. Se comprobó el correcto funcionamiento del sistema mediante una prueba piloto con cabras de raza Murciano-Granadina no productivas, alimentadas a nivel de mantenimiento. Posteriormente este sistema fue mejorado. Algunos de los cambios más importantes fueron la sustitución de la máscara por una urna en la que el animal introducía la cabeza entera, y el desarrollo de un software que registraba y guardaba de forma automática las concentraciones de O_2, CO_2 y CH_4 del aire espirado. Esta mejora permitía medidas de gases durante periodos de tiempo más largos y el registro de muchos más datos. Estas modificaciones también fueron validadas mediante una prueba piloto con ovejas de raza Manchega secas.

Posteriormente se realizaron tres experimentos. En todos ellos las dietas fueron raciones mixtas que diferían en la inclusión de cereal o subproductos fibrosos. Se estudió el efecto de la dieta sobre la digestibilidad, balances de energía y carbono-nitrógeno, oxidación de los nutrientes, parámetros del rumen, producción de metano y, en su caso, producción y composición de la leche. En el primero de los experimentos se utilizaron ovejas de raza Guirra secas y no gestantes y se observó que la sustitución del cereal por subproductos fibrosos no tuvo efecto negativo sobre el metabolismo energético y resultó en balances de energía positivos y similares (62 kJ/kg $PV^{0,75}$, de media). En los otros dos experimentos se utilizaron cabras de raza Murciano-Granadina en mitad de lactación. En el primero de ellos la dieta no afectó el balance de energía, que fue positivo, con un valor medio de energía metabolizable ingerida de 1.444 kJ/kg $PV^{0,75}$. En el siguiente experimento las cabras presentaron balances de energía negativos, que se acentuaron con la sustitución del cereal por los subproductos fibrosos. El caso más desfavorable fue la dieta más fibrosa con una oxidación de la grasa del 74,4% respecto a una oxidación de los carbohidratos del 18,5%. También la producción de metano fue superior con las dietas fibrosas (34,8 g/d, de media) que con la dieta de cereal (24,7 g/d). En ambos estudios con cabras en lactación no se observó

efecto del tipo de dieta sobre la producción de leche; en cambio, el contenido de grasa de la leche se incrementó, al igual que la producción de ácido acético en el rumen.

La determinación del factor de calibrado para el O_2 (1,005 ± 0,0101) confirmó el buen funcionamiento del equipo. Por otro lado, las pequeñas diferencias entre la producción de calor obtenida mediante calorimetría indirecta y el balance de carbono-nitrógeno (2% en ovejas y 1% en cabras) demostraron que este sistema permite determinar la producción de calor de los animales de forma fiable y precisa.

La emisión de metano de ovejas secas y no gestantes fue de 18.2 g/d y la de cabras en lactación fue de 30.7 g/d. Además, en los trabajos de esta Tesis se han estimado las necesidades energéticas de mantenimiento en dos razas de ovejas autóctonas españolas, como son las razas Guirra y Manchega, mediante regresión lineal o medición de la producción de calor en ayunas, respectivamente; las necesidades netas de mantenimiento fueron 270 kJ/kg $PV^{0,75}$, de media. En el caso del ganado caprino de raza Murciano-Granadina, en mitad de lactación, la eficacia media de utilización de la energía metabolizable para la lactación fue de 0,61.

RESUM

Des de fa anys s'ha tractat de conèixer les necessitats energètiques dels remugants a fi de formular racions ajustades, però s'ha comprovat que hi ha una gran varietat de factors que els afecten; per això són necessaris estudis que avaluen l'efecte d'estos factors. Com a conseqüència, el principal objectiu d'aquesta Tesi va ser dissenyar i validar un equip de respirometría, basat en calorimetria indirecta, que permetera avaluar les necessitats en energia de menuts remugants de forma precisa. Es va pretendre des de l'inici que fóra un sistema mòbil i de relatiu baix cost. A més, a este sistema també se li va incorporar un analitzador de gas metà, que permetia el mesurament de les emissions d'este gas d'efecte hivernacle i la quantificació de les pèrdues energètiques en forma de metà.

Inicialment l'equip tenia connectada una màscara que es col·locava en la cara de l'animal. Una mostra del gas espirat era emmagatzemada en una bossa d'arreplega de gasos que era connectada a l'analitzador, el qual mesurava la concentració d'O_2, CO_2 i CH_4 de l'aire. Es va comprovar el funcionament correcte del sistema per mitjà d'una prova pilot amb cabres de raça Murciano-Granadina seques, alimentades a nivell de manteniment. Posteriorment este sistema va ser millorat. Alguns dels canvis més importants van ser la substitució de la màscara per una urna en què l'animal introduïa el cap sencer, i el desenrotllament d'un programari que registrava i guardava de forma automàtica les concentracions d'O_2, CO_2 i CH_4 de l'aire expirat. Esta millora permetia mesures de gasos durant períodes de temps més llargs i el registre de moltes més dades. Estes modificacions també van ser validades per mitjà d'una prova pilot amb ovelles de raça Manxega seques.

Després es van realitzar tres experiments. Les dietes van ser racions mixtes que diferien en la inclusió de cereal o subproductes fibrosos. Es va estudiar l'efecte de la dieta sobre la digestibilitat, balanços d'energia i carboni-nitrogen, oxidació dels nutrients, paràmetres del rumen i producció de metà i, en el seu cas, sobre els rendiments productius. En el primer dels experiments es van utilitzar ovelles de raça Guirra seques i no gestants i es va observar que la substitució del cereal per subproductes fibrosos no va tindre efecte negatiu sobre el metabolisme energètic i va resultar en balanços d'energia positius i semblants (62 kJ/kg $PV^{0,75}$, de mitja). En els altres dos experiments es van utilitzar cabres de raça Murciano-Granadina en mitat de lactació. En el primer d'ells la dieta no va afectar el balanç d'energia, que va ser positiu, amb un valor mitjà d'energia metabolitzable ingerida de 1.444 kJ/kg $PV^{0,75}$. En el següent experiment les cabres van presentar balanços d'energia negatius, que es van accentuar amb la substitució del cereal pels subproductes fibrosos. El cas més desfavorable va ser la dieta més fibrosa amb una oxidació del greix del 74,4% respecte a una oxidació del carbohidrats del 18,5%. També la producció de metà va ser superior amb les dietes fibroses (34,8 g/d, de mitja) que amb la dieta de cereal (24,7 g/d). En ambdós estudis amb cabres en lactació no es va observar efecte del tipus de dieta

sobre la producció de llet; en canvi, el contingut de greix de la llet es va incrementar, igual que la producció d'àcid acètic en el rumen.

La determinació del factor de calibrat per a l'O_2 (1,005 ± 0,0101) va confirmar el bon funcionament de l'equip. D'altra banda, les xicotetes diferències entre la producció de calor obtinguda per mitjà de calorimetria indirecta i el balanç de carboni-nitrogen (2% en ovelles i 1% en cabres) van demostrar que este sistema permet determinar la producció de calor dels animals de forma fiable i precisa.

L'emissió de metà de ovelles seques i no gestants fou de 18.2 g/d i la de cabres lactants fou de 30.7 g/d. A més, en els treballs d'esta Tesi s'han estimat les necessitats energètiques de manteniment en dos races d'ovelles autòctones espanyoles, com són les races Guirra i Manxega, per mitjà de regressió lineal or mesurament de la producció de calor en dejú, respectivament; les necessitats netes de manteniment van ser 270 kJ/kg $PV^{0,75}$, de mitja. En el cas del bestiar caprí de raça Murciano-Granadina, en mitat de lactació, l'eficàcia mitjana d'utilització de l'energia metabolitzable per a la lactació va ser de 0,61.

GENERAL INTRODUCTION

A BRIEF HISTORY OF CALORIMETRY

Energetic metabolism measurements have been a subject of research since the eras of Leonardo da Vinci (1452–1519). Early thinking about air, fire, and life was marvelously unformed. It was known since prehistory that animal life depended on air, but what air was and how it supported life was unknown. Leonardo da Vinci, in the *Codex Atlanticus,* stated that "*animals could not survive in an atmosphere that would not support a flame*", but the development and acceptance of the theory of combustion and metabolism was severely rejected and it took over 300 years until it was accepted. In the late 1700's, Antoine Lavoisier and Pierre Laplace designed and built the first animal calorimeter, and reported their breakthrough observations establishing the relationship between O_2 use, CO_2 production, and heat production. Lavoisier determined that an animal and a fire produced the same amount of heat per unit of carbon dioxide produced. From these and other philosophers and researchers, the generalization that life is primarily a combustion process was developed.

At the end of the nineteenth century, Rubner offered experimental evidence in support of Lavoisier's theory that energy exchange in the body acts according to the principle of the conservation of energy. He showed that different nutrients produce the same quantity of energy on combustion in the body as outside the body when the end products are the same. Rubner's results were later confirmed by Atwater and Armsby, who showed that the quantity of energy produced by complete combustion of the nutrients outside the body is equivalent to the quantity of energy leaving the body as heat, energy excreted with the feces, urine and combustible gases, and/or stored in the form of protein and fat. The results of those experiments are described in an equation which may be considered as the fundamental equation for the science of nutrition:

Energy intake = E. in feces + E. in urine + E. in methane + E. in heat + E. retained

This equation represents the use that the animal does of the dietary energy and the form in what the energy is lost or retained in the animal body. Gross energy intake by the animal less energy losses in feces, urine and methane is metabolizable energy (ME) and represents the amount of energy available for body functions (maintenance, growth, production of milk, etc.). However, ME is used at a tissue level with an efficiency (k) of less than 1, such that the conversion of ME to net energy is associated with the heat production. This quantity of heat, produced as a result of the chemical and physical processes associated with digestion of foods and metabolism of the nutrients derived from them, is known as heat increment. This varies considerably, depending on the composition of the diet, the amount of feed ingested and the various processes for which nutrients are used. Besides, it is difficult to determine accurately since cannot be measured directly, it is the difference of heat production obtained between a fed animal and this fasted animal.

In energy metabolism studies, heat produced by animals is measured as total heat production (HP), which includes the heat used for maintenance and wasted as heat increment. Thus, ME less HP will be retained energy (RE), which is the energy used for growth, milk production or pregnancy, either is stored in the body or leaves it as chemical energy. So, this last statement is based in first thermodynamics law, this is the law of conservation of energy and holds that energy can be transferred or transformed from one form to another but neither created nor destroyed. This law dictates acceptance of the equality: ME = HP + RE. Consequently, if two of these entities are measured, the third can be calculated by difference.

The ME value of a food is determined in a feeding trial similar to a digestibility trial, but in which urine and methane are collected as well as feces. Urine and feces are separated easily by metabolic cages, while the determination of methane production, like HP, requires more expensive, complex and precise equipment based in calorimetric techniques. RE can be determined directly according several methods, as we shall describe below. So, the direct determination of RE is not easy due to the complexities involved in the methods, and RE values used to formulate diets are usually only estimates and substantial errors can exist in the estimates.

CALORIMETRY

As just discussed, to realize studies of energetic metabolism is necessary to measure the energy and this can be determined by calorimetry. Calorimetry means the measurement of heat and is based in the same laws that the bioenergetics, i.e. first and second thermodynamics laws. The first law of thermodynamics is the law of conservation of energy, mentioned above, and second law of thermodynamics states that all forms of energy are quantitatively convertible to heat.

So, the partition of food energy in the animal can be known according to these laws; if the ME intake of an animal is known, then the measurement of its HP will allow calculate its energy retention. The HP of animals can be measured physically using a procedure known as direct calorimetry. Alternatively, HP can be calculated from the respiratory exchange of the animal by indirect calorimetry.

Direct calorimetry

Direct calorimetry is simple in theory, but difficult in practice. Calorimeters designed to measure the heat production of an animal are based on the same general principle as the bomb calorimeter, in that the heat evolved is used to increase the temperature of a surrounding medium. The animal calorimeter is an airtight insulated chamber, in which oxygen is supplied by flow of air and heat loss from the animal can therefore be measured directly.

Lavoisier and Laplace were the first to measure animal heat production. The animal was enclosed in a chamber surrounded by ice, and the amount of ice melted within a given period of time, multiplied by the melting heat of the ice (330 kJ/kg), was recorded as the measure of the heat given off by the animal. Provided that all the heat produced was free heat, it would be equivalent to the HP of the animal. Nowadays, direct calorimetry is measured using more sophisticated instrumentation, but the principle remains the same. In the gradient layer calorimeter, the latest, the quantity of heat is measured electrically as it passes through the wall of the chamber. This type of calorimeter lends itself to automation, and both sensible (i.e. those with radiation, conduction and convection) and evaporative heat losses can be recorded automatically. Although, because animal direct calorimeters are expensive to build and earlier types required much labour to operate them, animal calorimetry today is mainly carried out by the indirect method.

Indirect calorimetry

Indirect calorimetry is based on the principle that metabolic heat production is mainly the result of oxidation of carbohydrates, fats and proteins. Thus, if all compounds were completely oxidized, HP could be readily calculated from the amounts of O_2 consumed and CO_2 produced. However, in the animal, incomplete oxidation of protein results in combustible nitrogenous compounds (primarily urea) which are excreted in the urine. In addition, in the case of ruminants, anaerobic fermentation yields combustible gases, primarily CH_4. The general equation that resumes all it and by which HP is estimated is Brouwer equation (Brouwer, 1965):

$$HP = 16.18 \times O_2 + 5.02 \times CO_2 - 2.17 \times CH_4 - 5.99 \times N$$

where HP is heat production (kJ), O_2 is the rate of oxygen consumption (L), CO_2 is the rate of carbon dioxide production (L), CH_4 is the rate of methane production (L) and N is the nitrogen excreted in the urine (g).

Indirect calorimeters may be of closed circuit or open circuit type. In the closed circuit system calorimeter the animal is enclosed in a temperature controlled chamber. The oxygen used by the animal is replaced by a metered supply of pure O_2. The total oxygen consumption of the animal is determined by volumetric measurement of the oxygen supplied. The air in the chamber is continuously circulated through an absorbent which removes water and CO_2. Thus, the amount of the produced CO_2 is found directly by weighing the absorbers before and after the experiment and calculating the difference. CH_4 is allowed to accumulate within the chamber and its production is calculated as the concentration difference between the beginning and end of the test period times the volume of the system. Corrections must be made for the composition of the air in the respiration chamber before and after the experiment. Other disadvantages of this system are that since the air is pumped through the

chamber, it must be completely airtight and under constant supervision. Moreover, continuous recording of the CO_2 and CH_4 production is not possible.

Because of those disadvantages, the most common type of calorimeter is the open circuit indirect calorimeter. In this type of system, a mask, head hood or animal chamber may be used. Air is drawn past the animal at a precisely determined rate. O_2, CO_2 and CH_4 concentrations must be accurately determined in both the incoming and outgoing air. Rates of consumption or production of these gases are calculated as the difference in concentration between incoming and outgoing air times the flow rate. This type of system is susceptible to error because of the high degree of accuracy required in the measurement of air flow and gas concentrations. But continuous recording of the O_2, CO_2 and CH_4 contents in the outgoing air are permitted by head hood or animal chamber.

According Lachica and Aguilera (2008), any out-door method to estimate energy metabolism must fulfill certain requirements: validity, reliability, acceptability, accuracy and cost. The four first requirements are widely accomplished in a calorimetry lab but with high economical cost. When cost has to be low, still there are options to accomplish the other requirements at the expense of a higher labour cost, an option is a mobile open-circuit respirometry system. To obtain rapid response times in short periods, masks or head hoods in connection with open-circuits have been frequently employed in ruminants (Brosh, 2007). The system can be incorporated to a cart to obtain a mobile system.

A mask encloses only the mouth of animals and it can be incorporated to a mobile system and be able for monitoring many animals near to their normal environment wherever there is a power source. However, the mask prevents animals from eating during the measurements and its prolonged use may cause discomfort and distress, and consequently can affect the gas measurements. So, masks are appropriate only for short periods of measuring. Therefore, for long terms measures, the use of head hood is preferred to mask. Various types of head hood systems have been used with degrees of success in a number of nutritional and physiological studies (Kelly *et al.*, 1994; Nicholson *et al.*, 1996; Takahashi *et al.*, 1999; Suzuki *et al.*, 2008). The head hood involves the use of an airtight box that encloses the animal's head. Hoods should be designed to provide sufficient feeding space and enough room for the animal to move its head in an unrestricted way. However, dead space must be minimized to enable the equipment to respond quickly to changes in rate of gaseous exchange and animal metabolism (Takahashi *et al.*, 1999). A sleeve is placed around the animal's neck in the rear panel of the hood to minimize air leakage (Kelly *et al.*, 1994) and a clear window or door at the front to facilitate feeding and watering. Compared to the face mask system, the ventilated hood system is advantageous for conducting the gas exchange measurements throughout the day and even during eating; further, it is less stressful for the animal.

OTHER METHODS TO ESTIMATE THE HEAT PRODUCTION

Carbon and nitrogen balance

The energy is mainly stored by the growing and fattening animal as protein and fat, since carbohydrates reserves of the body are small and relatively constant. Estimates of carbon (C) and nitrogen (N) balances of the animal can be obtained by determining of C and N contents ingested with the feed and both excreted in feces and urine, in addition to C eliminated as CO_2 and CH_4. Consequently, calorimetry is necessary to obtain respired gases. The RE can then be obtained by multiplying the quantities of nutrients stored by their calorific values. Once the RE calculated, HP can be estimated by difference of ME minus RE. This procedure is known as the C and N balance technique.

The advantages of the C and N balance technique are that no measure of oxygen consumption is required, though CH_4 and CO_2 production is need to measure. Moreover, energy retention is subdivided into that stored as protein and that stored as fat.

Comparative slaughter

Other method that measures RE directly as C and N balance, but in this case not do use of calorimetry, is the comparative slaughter technique. In this procedure a uniform group of animals are fed a common ration for a minimum of two weeks. At the end of the adaptation period, a sample of the animals is slaughtered and the body energy content is determined. The remaining animals undergo predetermined treatments for a period of time, and are then slaughtered and energy content of the bodies is determined. The RE is then calculated as the difference in body energy contents between the initial and final slaughter groups.

This technique has advantages over the calorimetric techniques because they usually allow experiments to be conducted under situations more similar to those found in the livestock industries and requires no elaborate apparatus. But this technique is expensive, laborious and destructive (i.e., an animal can be used only once).

More methods

The search for non-destructive and inexpensive methods of estimation of body composition, and consequently the determination of RE and HP, has led to the evaluation of numerous methods including various water dilution procedures, ultrasonic scanning, [40]K counting, three dimensional photography, nuclear magnetic resonance and computer assisted tomography.

COMPARISON OF CALORIMETRY WITH OTHER METHODS

In the literature, indirect calorimetry is quite often used as a reference for other methodologies for the measurement of heat production. However, several authors have challenged its validity by comparing an energy balance with its heat component measured by indirect calorimetry or respiratory quotient method (RQ) against the C-N balance (CN), or against the comparative slaughter technique (CS). When performing a C-N balance, the N balance is measured by conventional methodology, comparable to those often used in combination with RQ. Measurement of the C balance requires quantitative measurement of all C inputs (food) and losses (feces, urine, CH_4, CO_2), using similar equipment as indirect calorimetry, with the exception of O_2 analyzers. Energy and protein balances can be estimated to attain RE, and heat derived by difference between ME intake and RE. In this way, the assumptions underlying the Brouwer equation or equivalents are avoided, and within-day patterns of heat can be examined.

The latter is not possible using the CS technique, typically performed in growing subjects, preferably over a rather large weight range. The CS technique measures protein, fat and energy retention by difference between their respective quantities at the end and start of the measurement. For estimating starting protein, fat and energy mass at the start, a representative initial group of animals is sacrificed. Metabolizability of the ingested energy has to be measured and considered representative for the entire growth trajectory. Production of combustible gases is usually ignored. It is generally acknowledged that the CS technique is most reliable, but any errors made would typically lead to underestimation of the energy retained, whereas errors in the methods based on gaseous exchange would point in the opposite direction. From the comparison of RQ with CS it appears that there may be a systematic overestimate of energy retained by RQ, but experimental contrasts are generally well predicted. On the other hand, as CS has to be conducted over a large range in body weights and RQ is usually performed over a week or less (quite often respiration measurements are done over a 24 or 48 h period), it is likely that part of the difference presented in literature, attributed to methodological errors, may actually reflect a real difference in heat production related to slightly different or varying housing conditions.

The advantages of indirect calorimetry and C-N balance, in comparison with CS, are that they do not involve slaughtering of the animal, therefore, they can be repeated time and again with the same animal, and theoretically they can measure small retentions of energy during short intervals of time. Moreover, RQ and CN methods show quite comparable results, usually leading to slightly higher estimates of heat production by RQ, leading to lower estimates of energy retention. Thorbek (1975), Christensen *et al.* (1988) and Zhao *et al.* (1997) concluded from their comparison of RQ and CN methods that, from a discussion of sources of errors, there is no reason to prefer one over the other, but it should be noted that the obvious advantage of RQ over CN is that RQ method provides a good lead to estimate the net rate of substrate

oxidation. Therefore, heat can be predicted satisfactorily from the O_2 consumed and CO_2 produced using factors derived from the oxidation of carbohydrates, fat and protein. A critical attitude towards this approach is required when estimate short term variation in heat production and animals are in strenuous exercise or an environmental challenge, which energy containing intermediates metabolites (e.g. ketone body, urea) can be temporarily stored in the body and it is not completely oxidized.

Of all the methods to determine heat production in ruminants, indirect calorimetry seems to outperform the rest. In the past, the complexity and cost of the apparatus required for animal calorimetry limited its use to a small number of nutritional research establishments. Improved funding of research has gradually removed this restriction, but even so, animal calorimetry remains a specialized topic and few nutritionists become involved in it.

FEEDING SYSTEMS

Since the middle of the 19th century, scientists have attempted to devise feeding systems that would allow farmers to calculate appropriate rations for their domestic animals. All Feeding systems seek to match the supply of feed energy to the energy requirements of the animal. These systems usually provide sets of tables that give respectively the energy requirements of the animals for their different physiological states (maintenance, growth, gestation or lactation) and the energetic value of the feeds; both are expressed according to the same feed evaluation system.

Various Feeding systems have been published for ruminants in world, which are classified in two groups, ME or NE Feeding systems. There is no difference in principle between the ME and NE systems, with both systems recognizing that the energy requirement of ruminant animals is the sum of their energy requirements for maintenance and production. The NE concentration in the diet is the basal unit for both systems; the only difference between these systems is where the energetic efficiencies (k) are embodied within the calculation. In the ME system the energetic efficiencies are used for ration formulation and the prediction of animal performance, while in the NE system the efficiencies are included as part of the energy evaluation of feeds.

If efficiency of utilization all nutrients for all biological processes was the same, then ME would be just as accurate as NE (all we would be doing is multiplying by a constant). But it was long recognized that dietary ME is used with differing efficiencies depending on source, intake level, and function for which it is used by the animal. These different efficiencies created substantial problems with determining the nutritive value of feedstuffs and quantitatively expressing the results as a single value.

For instance, the Agricultural Research Council (ARC, 1980) is a ME Feeding system which is based on calorimetric studies and ME is determined at maintenance. On the other hand, the National Research Council (NRC, 1981) is a NE Feeding system and their

values are based in comparative slaughter studies in which animals were housed outdoors and fed at least two levels of feed above maintenance. The efficiency varies directly with the ME concentration in the diet; so, for 9 and 12 MJ/kg dry matter, predicted k for maintenance in beef cattle (NRC, 1996) are 0.60 and 0.68 respectively, however the corresponding k for maintenance obtained by calorimetry (e.g., ARC, 1980) are 0.69 and 0.74, respectively. Larson and Johnson (1997) also reported greater efficiencies of use of energy for body weight gain from calorimetry experiments than from slaughter experiments. This is in agreement with Quiniou *et al.* (1995) and Birkett and de Lange (2001) who stated that the calorimetry technique typically gives higher estimates for energy retention than does the comparative slaughter technique, as we discussed earlier. Therefore, influence of activity, feeding level and diet quality are implicitly and explicitly accounted for in the estimates of the energy value of the feed and in the estimates of animal requirements by comparative slaughter, as well as other energy losses not accounted for in short-term calorimetry studies (e.g. hair loss). Besides, some differences between the two systems are inherent because of the different databases that were used to develop the systems.

No currently available system yields accurate estimates, even within a single species, under different productive conditions, and no current system has universal applicability. Due to the advance of computer science, electronic engineering and gas analyzers technology, more effort would be necessary to quantify energy cost of domestic animal under different productive situations. Further strength in this topic is necessary and this Thesis attempt to raise the available information on energy metabolism for small ruminants.

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OBJECTIVES

The main objective of this Thesis was to design and validate a mobile open-circuit indirect calorimetry system for small ruminants. This system permits the rapid determination of CH_4 and CO_2 productions, and O_2 consumption and, thereafter, heat production for use in energy metabolism studies. Secondarily, this system can be used for measurement of gaseous emissions arising from enteric fermentation and related with greenhouse gas emissions. The unit is a functional respirometry system that combines mobility, ease of calibration and operation, and reasonable cost.

The first experiment describes the design of mobile open-circuit respirometry system, which is connected to a face mask adapted for small ruminants. In this paper the system was validated with a pilot experiment with goats for comparing the HP obtained by RQ method (calorimetry) and the CN method (C and N balances).

In second experiment is described the improvement made to previous mobile open-circuit respirometry system, in which the face mask and collecting bags for air sampling were replaced by a head hood and computerized acquisition and recording data for gases and air flux. Besides, a pilot experiment with sheep served to validate the new system, in which HP obtained by RQ method (calorimetry) and the CN method (C and N balances) was compared.

The third experiment discusses the effect of two diets, substituting dietary barley grain by soybean hulls and corn gluten feed blend on energy partitioning, substrate oxidation and C and N balance in non pregnant and no lactating Guirra ewes.

The fourth experiment evaluates the effect of substituting dietary corn grain by soybean hulls and corn gluten feed blend on energy partitioning, substrate oxidation and milk performance in Murciano-Granadina goats during mid lactation.

Finally, the fifth experiment examines the effect of replacement dietary corn grain by dry citrus pulp or soybean hulls on energy partitioning, substrate oxidation, C and N balance and milk performance in Murciano-Granadina goats during mid lactation. Moreover, this study also shows the effect of diet about methane emissions of lactating goats.

EXPERIMENT I

Description and function of a mobile open-circuit respirometry system to measure gas exchange for small ruminants

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ABSTRACT

A mobile open-circuit respirometry system was constructed for estimating heat production (HP) in small ruminants from CH_4 and CO_2 production and O_2 consumption by the respiratory quotient (RQ) method. The method was evaluated against HP estimates by C-N balance measurements (CN method). Calibration factors were established injecting N_2 into the system. Repetitive and consistent values for the calibration factor were obtained (1.0065 $\pm$ 0.01142, n = 9), which confirms the absence of leaks and good performance of the whole system. A pilot experiment with 14 Murciano-Granadina female dry goats was conducted to evaluate the system. The metabolizable energy intake was close to metabolizable energy for maintenance. Average HP measured by RQ method was in agreement with the average HP determined from CN method accounting for 505 and 500 kJ/kg $BW^{0.75}$/d, respectively. It is concluded that the mobile system was suitable for measuring gas exchange and energy metabolism in small ruminants.

Keywords: gas exchange, heat production, indirect calorimetry, mobile open-circuit.

INTRODUCTION

There is a renewed interest in the measurement of energy metabolism based on open-circuit respirometry as indirect consequence of the concern to reduce the livestock greenhouse emissions (*e.g.* ruminants contribute about 15% to global atmospheric CH_4 fluxes; Moss *et al.*, 2000). The open-circuit respirometry is an indirect calorimetry method that consists in measuring the gas exchange associated with the oxidation of energy substrates and determining the associated heat production (HP).

Any out-door method to estimate energy metabolism needs to fulfill certain requirements: validity, reliability, acceptability, accuracy and cost (Lachica and Aguilera, 2008). They are widely accomplished in a calorimetry lab but when cost has to be low, an option is a mobile open-circuit respirometry system. To obtain rapid response times in short periods, masks or head hoods in connection with open-circuits have been frequently employed in ruminants (Brosh, 2007). The system can be incorporated to a cart for monitoring animals near to their normal environment.

The objectives of the present study deals with the construction, function and evaluation of a mobile open-circuit respirometry system intended for use in energy metabolism studies that can also be used out-door. It is described as well a pilot experiment with goats for comparing the HP obtained by the RQ and the CN method.

MATERIALS AND METHODS

Open-circuit respiration system

It was built at the Polytechnic University of Valencia. It shows the particularity that no recording device was required to obtain the total average gas concentration reducing considerably the cost nevertheless with a high performance level. The instrumentation was installed on a mobile cart to make the system portable (Figure 1.1). Dimensions of the aluminum cart were 95 long × 50 wide × 95 cm high, with four 30 cm diameter wheels with inflated tires for absorbing land irregularities. Pipes, rotameter, flowmeter, air volume totalizer and fan were mounted on the bottom of the cart leaving the upper part free to allocate both the non-diffusing bag for gas sampling and, as optional, the gas analyzer unit. Collection bags were kept and lately transported to the lab for gas analysis, when gas analyzer equipment was on the top of the cart, analysis was done in situ.

The respirometry system has two separate sampling lines. The main line sucked air through a face mask made with a plastic funnel (15 × 2.5 inner diameter, and 25 cm long) with an internal rubber draught excluder glued to the internal border for the best fit of the animal face and it was fixed by mean of a rubber band around the head. The funnel was attached to a 2.5 cm (inner diameter) corrugated polyvinyl chloride (PVC) tube equipped with an air filter to keep away the dust.

Total air flow through the system was measured by a mass flowmeter with a range from 0 to 10,000 L/h (thermal mass flowmeter Sensyflow VT-S, ABB, Alzenau, Germany) and regulated by a manual valve at 3,000 L/h. Air suction was done by a centrifugal fan (CST60 Soler Palau Inc., Parets del Vallès, Barcelona, Spain) with 310 m³/h as maximum capacity situated at the end of the main sampling line with free escape for the air. Total flow was totalized (totalizer VT-S, ABB, Alzenau, Germany) and screen (C150 Universal Process Indicator, ABB, Alzenau, Germany) displayed. Mass flowmeter had digital output for computer connection. A subsampling line (polyethylene tubing, 5 mm inner diameter) was situated after the mass flowmeter to take a sample of gas from the main line by means of a membrane pump (ABB, Alzenau, Germany) of 250 L/h capacity. It was attached to a rotameter (DK800, ABB, Alzenau, Germany) with valve to set up the desirable flow rate into a 15 L non-diffusing gas collection bag (PanLab S.L., Harvard Apparatus, Cornellá, Barcelona, Spain).
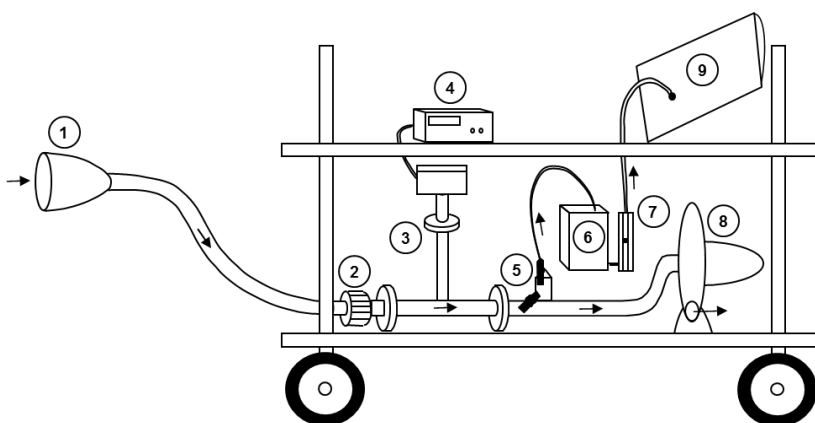
Figure 1.1. Mobile open-circuit respirometry system: (1) mask; (2) air filter; (3) mass flowmeter; (4) totalizer; (5) manual valves; (6) membrane pump; (7) rotameter; (8) centrifugal fan; (9) non-diffusing bag for air collection.

The non-diffusing collection bag containing the gas from the sampling period was connected by mean of a tube (5 mm inner diameter) to a membrane pump (ABB, Alzenau, Germany) and a rotameter (DK800, ABB, Alzenau, Germany) with a range from 10 to 100 mL/h, and to a gas analyzer unit. Previously, the gas sampled was filtered and dried passing through a 500 mL flask containing silica gel (Figure 1.2). The gas analyzers were combined in the gas analyzer unit (Easyflow 3020 model, ABB, Alzenau, Germany). The CH_4 and CO_2 were measured using infrared principle with a concentration range from 0 to 0.15% and 0 to 1.5%, respectively; O_2 was measured using paramagnetic principle from 19 to 21% and it was equipped with an atmospheric compensation module to account changes in pressure. The unit was an autocalibrated

model and no master cylinders were required. It was monitored with a digital panel meter and had digital output for computer connection.
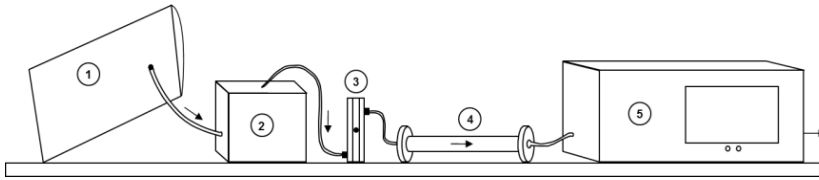
Figure 1.2. Gas analyzer equipment: (1) non-diffusing bag with the air collected; (2) membrane pump; (3) rotameter; (4) silica gel flask; (5) gas analyzer unit.

Whole system calibration

The whole system was calibrated injecting N_2 (99.99%) into the mask (McLean and Tobin, 1987) determined gravimetrically using a precision scale. Calibration factor was calculated according to Brockway *et al.* (1971).

Calculations

The CH_4 and CO_2 production and O_2 consumption were calculated as described Aguilera and Prieto (1986). An initial atmospheric air was collected and the gas concentrations were used as reference in calculations.

Pilot experiment

Animals and feeding

The experimental procedure was approved by the Committee on Animal Use and Care at the Polytechnic University of Valencia (Spain).

Fourteen Murciano-Granadina female dry goats of similar body weight (39.3 ± 0.69 kg BW) were used to determine the energy and C-N balances as well as the fasting heat production (FHP). Diet was 65 forage (alfalfa hay):35 concentrate (corn and green peas), and a vit-mineral mix. Its chemical composition was 89.3% for dry matter (DM); 25.9, 5.0, 39.6 and 17.1% for acid detergent fiber (ADF), lignin, neutral detergent fiber (NDF) and crude protein (CP), respectively; and 18.8 MJ/kg DM for gross energy (GE). The feeding level was close to the metabolizable energy (ME) for maintenance and with free access to water. Half daily ration was offered at 08:00 and 16:00 h, respectively.

Energy and C-N balance, and HP determination

Goats were allocated on individual metabolism cages at thermoneutrality. After 10 days of adaptation, feed intake, and total fecal and urine output were recorded daily for each goat during a 5 days period, as well as BW at the beginning and end of the period. Representative samples of diet, feces and urine was collected daily, stored at −20 ºC, and pooled for analysis. 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 39.54 kJ/L; Brouwer, 1965).

Within the balance trial, gas exchange was measured during 15 min/h per goat and repeated hourly from 09:00 to 21:00 h, in other words, 4 goats/h were sampled during the 12 h period (4 goats/d). The values obtained per goat were averaged and extrapolated to 24 h. The schedule was repeated after 3 days fasting, when balance trial was over, to determine FHP.

The HP (RQ method) measurements were averaged and expressed per day, and it was calculated according to Brouwer (1965) for O_2 consumption, CO_2 and CH_4 production (L), and urine N (N_{urine}, g) as HP (kJ) = 16.18 × O_2 + 5.02 × CO_2 − 2.17 × CH_4 − 5.99 × N_{urine}. Respiratory quotient (RQ) was calculated as CO_2 produced/O_2 consumed. Retained energy (RE) was calculated as the difference between MEI and HP.

The C-N balance (CN method) depends on measurements of C and N intake and their losses as urine, feces and gases (CO_2 and CH_4). The RE (kJ) was calculated according to Brouwer (1965) from the C (g) and N (g) balance (RE = 51.8 × C − 19.4 × N). The HP was calculated as difference between MEI and RE.

Data were analyzed by using the repeated-measures analysis of variance to determine the method effect. Tukey test was used to ascertain the statistical significance of differences for HP ($P < 0.05$).

Chemical analysis

DM was analyzed according to the methods of AOAC (2000). The GE content was determined in an adiabatic calorimetric pump. The NDF was 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. The ADF (via ANKOM) and lignin were determined according to Robertson and Van Soest (1981). Both NDF and ADF are reported exclusive of residual ash. 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.

RESULTS

The average value obtained for the calibration factor was 1.0065 ± 0.01142 (n = 9).

Table 1.1 shows the daily energy balance and HP determined by the RQ method. It is displayed the average MEI and the resultant RE, also the FHP and the energy loss as CH_4. The average RQ for fed and fasting goats were 0.97 and 0.73, respectively.

The daily C–N balance and the calculated RE and estimated HP by the CN method are also displayed in Table 1.1.

Table 1.1. Daily energy and C-N balances, heat production and retained energy calculated by the RQ and CN methods and fasting heat production of female dry Murciano-Granadina goats (n = 14).

		SEM[1]
RQ method [2] *(kJ/kg BW$^{0.75}$)*		
Gross energy intake	933.9	30.65
Energy in feces	299.7	16.58
Energy in urine	54.1	4.54
Energy in CH_4	34.3	4.11
MEI	545.9	21.94
HP	505.1	9.49
RE	40.7	6.61
Fasting HP	310.3	7.90
CN method [3] *(g/kg BW$^{0.75}$)*		
C intake	22.25	0.724
C in feces	7.15	0.371
C in urine	0.58	0.055
C in CO_2	12.81	0.347
C in CH_4	0.63	0.075
C retained	1.07	0.720
N intake	1.36	0.044
N in feces	0.35	0.019
N in urine	0.49	0.040
N retained	0.52	0.053
RE (kJ/kg BW$^{0.75}$)	45.6	6.71
HP (kJ/kg BW$^{0.75}$)	500.3	18.80

[1] SEM = standard error of mean.
[2] MEI = metabolizable energy intake; HP = heat production; RE = retained energy, calculated as RE = MEI - HP.
[3] RE = retained energy, calculated as RE = 51.8 x C retained – 19.4 x N retained; HP = heat production, calculated as HP = MEI - RE.

DISCUSSION

The accuracy of the gas exchange determination is further dependent on the ability of the system to measure gas composition and, mainly, the total volume passed through the system. The total air flow was determined by a calibrated thermal mass flowmeter that operates on the thermal principle, thereafter, the device determined the total volume directly avoiding corrections for pressure and temperature.

In addition to the low cost in comparison with a respirometry chamber, the open-circuit system attached to a face mask was chosen because it is best suited and sufficiently accurate to fast response applications for measurement of gas exchange of animals for short periods of time (Brosh, 2007). The mask prevents animals from eating during the measurements and its prolonged use may cause discomfort affecting the gas exchange. To avoid this inconvenience, face mask was placed for 15 min and the procedure repeated hourly during a 12 h period. Full adaptation of the animals was vital for the success of experiments.

A final check of the entire system was accomplished by releasing a known volume of N_2 to obtain calibration factors. The repetitive and consistent values confirmed the absence of leaks and good performance of the system.

The system was built for operation flexibility, it was feasible to be connected to a mask or a respirometry chamber and to measure a broad range of BW. Also, both the mass flowmeter and the gas analyzer unit had digital outputs for computer connections and data recording but to reduce considerably the cost, non-diffusing collection bags were used for sampling. The gas concentrations corresponded with the average gas exchange of the measuring period.

For comparison with literature data, FHP (Table 1.1) was also measured since the fasting reduces the effect of the diet on HP to a minimum. The agreement with published data can be considered as an index of the system reliability. Aguilera *et al.* (1991) with growing female Granadina goats found a value of 320 kJ/kg $BW^{0.75}$. The AFRC (1997) gave an average value of 315 kJ/kg $BW^{0.75}$. The RQ for fed and fasting goats were in close agreement with the regular values obtained under such circumstances and could be used as well as an index of reliability of measurements. For CH_4 production, Tovar-Luna *et al.* (2010) found an average value of 31 kJ/kg $BW^{0.75}$ for Alpine female dry goats fed near ME for maintenance.

Usually, the RE determined with the CN method is greater than the calculated with the RQ method and, thereafter, underestimates the HP with respect to RQ method (Blaxter, 1967). In our experiment, it was assumed that the HP determined by the RQ method could be overestimated since during daylight it is higher than during the night. However, both methods have given very close values (no significant differences; $P > 0.05$) accounting for 500 and 505 kJ/kg $BW^{0.75}$ for CN and RQ methods, respectively, in spite of the relatively short measuring periods (15 min) where the specific state of the animal can alter the regular gas exchange with respect to the usual 24 h measurements.

The agreement between both was another index of the absence of systematic errors. Discrepancies averaged only 0.9% when expressed as 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 in respirometry chambers for 24 hour fed at about maintenance obtained a discrepancy of 0.018.

CONCLUSIONS

The present study shows the suitability of a "homemade" open-circuit respiration system to measure CH_4, CO_2 and O_2 animal gas exchange and, thereafter, HP. The goats were easily adapted for short periods of sampling with no sing of discomfort or stress after an adaptation time. The system complies widely the accuracy requirement for determining gas exchange and, thereafter, HP. It could be adapted and used under a wide range of situations.

ACKNOWLEDGMENTS

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EXPERIMENT II

Low-cost mobile open-circuit hood system for measuring gas exchange in small ruminants: From manual to automatic recording

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ABSTRACT

Improvements of a homemade mobile open-circuit respirometry system for the rapid determination of CH_4 and CO_2 productions, and O_2 consumption and, thereafter, heat production (HP) for small ruminants are described and validated. Improvements consisted in three main features: utilization of a head hood (replacing the previous face mask); use of computerized control system, data acquisition and recording for gases and air flux (replacing collecting bags for air sampling); and use of a gas cooler to remove the air sample moisture (replacing the chemical drier (silica gel) approach). Calibration factors were established injecting N_2 and CO_2 in the system into the head hood. Repetitive and consistent values for the calibration factor were obtained for O_2 (1.0056 ± 0.00158, n = 5) and CO_2 (0.9924 ± 0.00915, n = 5) which confirmed the absence of leaks and the good performance of the system. In addition, an experimental test with 12 Manchega female dry sheep was conducted to validate the system. Three diets based on cereal grain (CGR), fibrous carbohydrates (FCH) and alfalfa hay (ALH) were used with four sheep per diet. Metabolizable energy intake (MEI) was close to metabolizable energy for maintenance. Average HP measured by indirect calorimetry (RQ method) was close to the average HP determined from carbon and nitrogen balances (CN method) accounting for 443 and 426 $kJ/kg^{0.75}$ body weight (BW) per day, respectively. Fasting HP was determined by the RQ method with one sheep from each diet accounting for 269 kJ/kg $BW^{0.75}$ per day. The head hood and computerized control, data acquisition and recording as well as the gas cooler improved the system by reducing the labour input without loss of functionality for measuring gas exchange and energy metabolism in small ruminants.

Keywords: gas exchange, indirect calorimetry, mobile open-circuit, head hood.

INTRODUCTION

Nowadays there is a big concern about gas production by ruminants in the scientific community. Many research institutions are building facilities for an accurate measure of such production in many cases with short economic budgets.

Usually, indirect calorimeters are associated with high cost facilities where respirometry chambers and equipment are allocated in a laboratory building. Any method developed to estimate the energy metabolism needs complying with some requirements: validity, reliability, acceptability, accuracy and cost (Lachica and Aguilera, 2008). The four first requirements are widely accomplished in a calorimetry lab but with high economical cost. When cost has to be low an option is to build a mobile open-circuit respirometry system where costs can be reduced drastically becoming an out-door method. A detailed description of a homemade mobile open-circuit respirometry system was published previously (Fernández *et al.*, 2012a). It showed the particularity that no recording apparatus was required to obtain the total average gas concentration during the measured period by using non-diffusing gas bags for air collection, and a face mask. Also, prior to gas analysis the air sampled was dried passing through silica gel to absorb the moisture.

To obtain rapid response time on relatively short periods, a face mask enclosing only the mouth of animals or head hoods in connection with the open-circuit have been employed before with good results (Brosh, 2007). However, face mask prevents animals from eating during the measurements and its prolonged use may cause discomfort and distress, therefore, the use of head hoods is preferred to masks for long-term measurements (Takahashi *et al.*, 1999). The computerizing for system control, data acquisition and recording requires low labour input and has the advantage of producing, if required, minute-to-minute records of gas exchange. Also, with respect to the labour reduction, the use of a chemical drier (*e.g.* silica gel) to absorb the moisture presents disadvantages since it needs to be often removed periodically instead of utilize a physics approach using a gas cooler.

The objectives of the present study deals with the evaluation of an improved system intended for use in energy metabolism studies: construction, function and technical approach. It is described the improvements made on a previous homemade mobile open-circuit respirometry system that can also be used out-door. It is described as well an experimental test with sheep using three different diets where HP determined by indirect calorimetry (RQ method) was compared with the estimated by C-N balance (CN method).

MATERIALS AND METHODS

Open-circuit respiration system

It was made and set up for small ruminants (sheep and goats). A detailed description of the homemade mobile open-circuit respirometry system was previously published (Fernández *et al.*, 2012a). Improvements made over this system consisted in the substitution of a face mask by a head hood, and collecting bags for air sampling by a computerized control system, data acquisition and recording for gases and air flux; as well as the substitution of the chemical drier (silica gel) approach to remove the air sample moisture by a physics one using a gas cooler.

As summary of the mobile open-circuit respirometry system, the instrumentation was installed on a mobile cart to make the system (Figure 2.1) portable to anywhere. Pipes, rotameter (DK800; ABB, Alzenau, Germany), flowmeter (Thermal Mass Flowmeter Sensyflow VT-S; ABB Automation Products GmbH, Alzenau, Germany), air volume totalizer (Totalizer VT-S; ABB Automation Products GmbH, Alzenau, Germany), adjustable and precise membrane pump (ABB Automation Products GmbH, Alzenau, Germany), gas cooler (SCC-C; ABB, Alzenau, Germany) and fan (CST60; Soler Palau Inc., Parets del Vallès, Barcelona, Spain) were mounted on the bottom of the mobile unit. The upper part allocated the gas analyzer unit for CH_4, CO_2 and O_2 measurement (Easyflow 3020; ABB Automation Products GmbH, Alzenau, Germany) and computer for system control, data acquisition and recording. All the original analytical apparatus were acquired with digital output for computer connection.

Figure 2.1. Whole mobile open-circuit respirometry system. Head hood attached to the front structure of a double metabolic cage with an animal on it.

The respirometry system had two separate but linked sampling lines. The main line suctioned air across a head hood attached to a corrugated polyvinyl chloride (PVC) tube (internal diameter 25 mm) equipped with an air filter to keep away atmospheric dust. A secondary line (internal diameter 5 mm), situated after de mass flowmeter, took a gas sample from the main line by means of the aforementioned membrane pump attached to the rotameter and connected to the gas analyzer unit. Previously, the gas sampled was filtered and dried passing through the gas cooler to remove moisture.

The head hood (Figure 2.2) was made with 1.5 mm galvanized plate (530 mm long × 1160 mm high × 360 mm wide; volume = 219 L). It was suspended on the front structure of the metabolic cage by two hooks placed on its rear side. The hood had a transparent acrylic (bolted and glued with silicon on the edge) window at the front (420 mm long × 530 mm high), and a drawer (500 mm long × 290 mm high × 350 mm wide) with a handle for pulling to open and place the animal food and water in a bucket. The drawer was locked by two lateral locks situated in its front side and main body of the head hood. A foam tape was placed on the edge of the drawer for an adequate seal. An opening (200 mm long × 520 mm high) was in the rear panel of the hood set up with a tightly woven nylon curtain (funnel shape) with a hole in the middle for the animal neck, fixed by four bolted platens and glued all around the opening edge. It was set up with a nylon drawstring through a fold edge to fit and tie it around the neck to avoid gas leaking. The animal was attached to the front structure of the metabolic cage by a necklace and chain so that it could freely stand or lie down. Atmospheric air entered into the head hood through an orifice (internal diameter 20 mm) made on its top on the opposite side of the main line suction air.

Figure 2.2. From left to right: front, lateral and rear side of the head hood.

System control, data acquisition and recording were handled by a computer (Fujitsu Siemens; Lifebook Series, Pentium 4 laptop, Munich, Germany) under a LabVIEW (www.ni.com/labview) environment. The software to operate the system was created

using National Instrument LabVIEW 7.1 (National Instruments, Austin, Texas, USA) running under Microsoft Windows XP. The system was capable to record data at intervals of 1 s, however, the data recording was set up at one/min. Gas analyzer unit and flowmeter was connected to the computer by an universal serial bus (USB) connector. A serial communication protocol was used with its programmable logic controllers (MODBUS Organization, Inc., Hopkinton, MA, USA; www.modbus.org) for communication between the analytical devices (analyser unit and flowmeter) and the computer. The electronics prototyping platform Arduino (www.arduino.cc) was used to send data from the gas analyser unit to LabVIEW by RS-232 protocol in real time. The flowmeter was monitored by a 10 bit analog to digital converter (model DS2438; Maxim Integrated Products, Inc. Sunnyvale, CA, USA).

Whole system calibration

The whole system was calibrated injecting pure gas N_2 (0.9999) and CO_2 (0.9999) into the head hood (McLean and Tobin, 1987) to produce an O_2 decrement and CO_2 increment. Calibration factors comparing the volume of gas injected and detected by the system were obtained. Total gas released was determined gravimetrically using a precision electronic scale (MOBBA mini-SP 0.2-30 kg). Sufficient gas was released (about 375 and 177 g of N_2 and CO_2, respectively, during 6 h) to give acceptable accuracy in the measurement of the change in cylinder weights, and injected at the required flow rate to simulate the O_2 and CO_2 exchange produced by an animal in the system. Calibration factors were calculated according to Brockway *et al.* (1971).

Calculations

Methane and CO_2 productions, and O_2 consumption were basically calculated as described Aguilera and Prieto (1986) using the Haldane transformation, except that no theoretical values for atmospheric CH_4, CO_2 and O_2 concentrations were used. Before gas measurement, atmospheric air was sampled and the gas concentration values used as reference in calculations.

Experimental test

Animals and feeding

Experimental procedure was approved by the Committee on Animal Use and Care at the Polytechnic University of Valencia (Spain).

Twelve Manchega female dry sheep of similar body weight (58 ± 1.2 kg BW) were selected to determine the energy and C-N balances. Three diets were used based on cereal grain (CGR), fibrous carbohydrates (FCH) and alfalfa hay (ALH) with four sheep

per diet. Cereal straw was offered as forage in CGR and FCH diet, and the concentrate was mixed and pelleted along with the premix. The forage:concentrate ratio (on dry matter basis; DM) of CGR and FHC diets was 30:70. Ingredients and chemical composition of the diets are shown in Table 2.1. The feeding level was close to metabolizable energy for maintenance (MEm). Sheep had free access to a vitamin-mineral block and water. Half daily ration was offered at 08:00 and 16:00 h, respectively.

Sheep were housed in a barn where temperature and relative humidity were automatically recorded every 15 min and averaged daily during the experimental trial; these ranged, respectively, from 20.2 to 25.1 °C and from 62 to 77%.

Energy and C-N balances, and HP determination

Sheep were allocated on individual metabolism cages. After 12 days of adaptation to experimental conditions, feed intake, total fecal and urine output were recorded daily for each sheep over a 5 days period. Body weight was recorded at the beginning and end of experimental period. Representative samples of diet, feces and urine were collected daily, stored at -20 °C, and pooled for chemical analysis. Metabolizable energy intake (MEI) was the difference between gross energy intake and energy losses in feces, urine and CH_4 (with an energy equivalent value of 39.54 kJ/L; Brouwer, 1965).

When the energy and C-N balances were done, gas exchange was sequentially measured during 24 h/sheep per diet. Gas exchange was repeated with one sheep from each diet after 3 days fast to measure the fasting heat production (FHP). Heat production (HP; kJ) determined by the RQ method was calculated according to Brouwer (1965) for O_2 consumption, CO_2 and CH_4 productions (L), and urine N (N_{urine}, g) as HP = 16.18 x O_2 + 5.02 x CO_2 - 2.17 x CH_4 - 5.99 x N_{urine}. Respiratory quotient (RQ) was calculated as CO_2 produced:O_2 consumed ratio. Retained energy (RE) was determined as the difference between MEI and HP. Retained energy (kJ) determined with the CN method was calculated according to Brouwer (1965) from the C (g) and N (g) balance (RE = 51.8 x C - 19.4 x N). HP was calculated as difference between MEI and RE.

Table 2.1. Ingredients and chemical composition of the diets based on cereal grain (CGR), fibrous carbohydrates (FCH) or alfalfa hay (ALH).

	Diets[1]		
	CGR	FCH	ALH
Ingredients (g/kg)			
Alfalfa hay	-	-	1,000
Barley	430.0	53.3	-
Straw	306.1	302.9	-
Soybean meal 44%	187.4	120.8	-
Soybean hulls	-	223.1	-
Gluten feed 18%	-	209.1	-
Wheat bran	-	48.3	-
Lard[2]	20.8	20.9	-
By-pass fat[3]	6.4	12.7	-
Beet molasses	27.8	3.5	-
Calcium carbonate	8.9	1.9	-
Bicalcium phosphate	6.9	-	-
Sodium chloride	2.9	0.8	-
Premix[4]	2.8	2.8	-
Chemical composition (% DM)			
Dry matter (%)	89.12	90.06	89.37
Ash	6.37	6.75	9.50
Crude protein	16.93	15.82	15.23
Ether extract	5.07	6.28	0.99
Neutral detergent fiber	43.66	50.77	56.48
Acid detergent fiber	16.73	26.22	38.94
Lignin	1.07	0.92	7.57
Carbon	42.79	42.45	44.93
Gross energy (MJ/kg DM)	18.60	18.73	17.75

[1] CGR = cereal grain diet; FCH = fibrous carbohydrates diet; ALH = alfalfa hay diet.
[2] Fused lard provided by VALGESS S.L., Carpesa, Valencia, Spain.
[3] By-pass fat of palm fatty acid distillate. Provided by Norel Animal Nutrition, Norel S.A. (Spain).
[4] Provided by NACOOP S.A. (Spain). Premix composition: Se 40 mg/kg; I 250 mg/kg; Co 80 mg/kg; Cu 3,000 mg/kg; Fe 6,000 mg/kg; Zn 23,400 mg/kg; Mn 29,000 mg/kg; S 60,000 mg/kg; Mg 60,000 mg/kg; vitamin A 2,000,000 IU/kg; vitamin D3 400,000 IU/kg; vitamin E 2,000 mg/kg; nicotinic acid 10,000 mg/kg; choline 20,300 mg/kg.

Chemical analysis

Feed and feces were dried in a forced air oven at 55 °C for 48 h and then grounded to pass 1 mm screen. Urine was dried by lyophilization. Chemical analyses were conducted according to methods of AOAC (2000) for dry matter (no. 934.01), ash (no. 942.05) and ether extract (no. 920.39). Gross energy content was determined in an adiabatic bomb calorimeter (Gallenkamp Autobomb; Loughborough, UK). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) of diets were determined by filter bags and a fiber analyzer (A220; ANKOM Technologies, Fairport, NY, USA) following AOAC (2000) official methods (no. 973.18) according to Mertens (2002). Lignin was determined according to Robertson and Van Soest (1981). C and N were analyzed by Dumas principle (TruSpec CN; Leco Corporation, St. Joseph, MI, USA). Multiplying N by a factor of 6.25 converted the results to crude protein.

Statistical analyses

Experimental data were analyzed by analysis of variance with the animal as experimental unit to determine the treatments (diets) effect. The effects of the diet (based on cereal grain, fibrous carbohydrates and alfalfa hay) on energy and C-N balances were analyzed using the PROC GLM of SAS (2001). The statistical model was Y = μ + α (D) + ε , where Y was the dependent variable, μ the overall mean, D the fixed effects of diet and ε the random error. Tukey multiple range test was used to ascertain the statistical significance of differences (P < 0.05). Mean values of HP obtained by RQ and CN method were compared by Fisher's least significant difference (LSD) test, and significance was set at P < 0.05.

RESULTS

The average value for the calibration factor was 1.0056 ± 0.00158 (n = 5) and 0.9924 ± 0.00915 (n = 5) for O_2 and CO_2, respectively.

Table 2.2 shows the daily energy and C-N balances for three diets. Gross energy, C and N intake of sheep from ALH diet was significantly higher than this of CGR and FCH diets. And energy, C and N losses in feces also were greater for ALH diet.

HP determined by the RQ method and the resultant RE are also displayed in Table 2.2, alongside calculated RE and estimated HP by the CN method.

The average RQ value for fed and fasting sheep was 0.9 and 0.7, respectively. Average FHP was 269 ± 10.3 kJ/kg BW$^{0.75}$ per day.

Table 2.2. Daily energy and C-N balances, heat production and retained energy calculated by indirect calorimetry (RQ method) and C-N balance (CN method) of female dry Manchega sheep (n = 12) according to the type of diet.

	Diets[1]			SEM[2]	P-value
	CGR	FCH	ALH		
Energy balance [3] (kJ/kg BW$^{0.75}$)					
Gross energy intake	785.5^a	801.0^a	1009.9^b	35.13	0.004
Energy in feces	212.1^a	213.6^a	444.6^b	34.00	0.001
Energy in urine	19.9^a	25.8^{ab}	33.8^b	4.17	0.045
Energy in CH_4	39.7^a	50.8^b	58.0^b	2.84	0.011
MEI	513.8^b	510.7^b	473.5^a	14.32	0.005
RQ method [4] (kJ/kg BW$^{0.75}$)					
HP	431.3^a	404.3^a	493.8^b	13.38	0.008
RE	82.6^b	106.5^b	-20.4^a	19.24	0.001
CN method [5] (g/kg BW$^{0.75}$)					
C intake	18.07^a	18.16^a	25.56^b	1.132	0.001
C in feces	4.15^a	4.39^a	12.26^b	1.146	0.001
C in urine	0.72^{ab}	0.50^a	0.92^b	0.096	0.042
C in CO_2	10.32^{ab}	9.63^a	11.42^b	0.275	0.013
C in CH_4	0.72^a	0.92^{ab}	1.05^b	0.051	0.011
C retained	2.17	2.71	-0.09	0.534	0.083
N intake	1.14^a	1.08^a	1.39^b	0.046	0.005
N feces	0.29^a	0.33^a	0.50^b	0.031	0.003
N urine	0.43	0.32	0.33	0.067	0.376
N retained	0.42	0.43	0.56	0.089	0.432
RE (kJ/kg BW$^{0.75}$)	104.3^b	132.0^b	-15.5^a	22.12	0.001
HP (kJ/kg BW$^{0.75}$)	409.6^a	378.7^a	489.0^b	28.01	0.007

$^{a-b}$ Means within a row with different superscripts differ (P < 0.05).
[1] CGR = cereal grain diet; FCH = fibrous carbohydrates diet; ALH = alfalfa hay diet.
[2] SEM = standard error of mean.
[3] MEI = metabolizable energy intake.
[4] HP = heat production; RE = retained energy, calculated as RE = MEI - HP.
[5] RE = retained energy, calculated as RE = 51.8 x C retained – 19.4 x N retained; HP = heat production, calculated as HP = MEI - RE.

DISCUSSION

DM intake of sheep from ALH group (1.18 kg/d) was greater than this of the other two groups (0.89 kg/d, on average for CGR and FCH); due to the type of diet: forage against mixed diet. The high content of fiber on ALH diet compared to CGR and FCH appeared to be the main factor responsible for the significantly higher losses in feces: such as energy, C and N.

HP was calculated by the open-circuit respirometry system described in this experiment. It has the primary function of measuring gas exchange and then the determination of HP based on the animal CH_4 and CO_2 productions, and O_2 consumption. The accuracy of the gas exchange determination is further dependent on the ability of the system to measure gas composition and, mainly, the total volume of the air moved through the respirometry system (Fernández *et al.*, 2012a). To obtain a fast response of the system on short time period, masks or hoods enclosing only, respectively, the animals face or head in connection with open circuits, have been frequently employed with ruminants (Young *et al.*, 1988; Takahashi and Young, 1992; Puchala *et al.*, 2007). The open-circuit system attached to a face mask presents the lowest cost and it is best suited and sufficiently accurate to fast response applications for measurement of gas exchange of animals for a short period of time (Fernández *et al.*, 2012a).

However, the mask prevents animals from eating and may cause discomfort and distress and consequently can affect the gas exchange. Also, the use of face mask requires a very close contact with the staff involved in the study for full adaptation of the animals to experimental conditions (Lachica and Aguilera, 2005). To avoid this inconvenience, the head hood represented the best and cheapest alternative to measure gas exchange for 24 h without disturbing the animal (Kelly *et al.*, 1994; Brosh, 2007). The system was built for operation flexibility which allows a variety of experimental arrangements. It was feasible to be connected not only to a head hood, but also to a mask or a respirometry chamber and is able to measure a broad animal range of BW.

A final check of the entire system was accomplished by releasing a known volume of N_2 and CO_2 from a cylinder. The repetitive and consistent values confirmed the absence of leaks and good performance of the whole system. The range of temperature during the experimental test implied that no HP associated with temperature stress was elicited.

For comparison reasons with literature data, FHP was also measured since the fasting reduces the possible effect of the diet on HP to a minimum. The agreement with published data can be considered as another index of the system reliability. FHP was in agreement with the value obtained for the same sheep breed by using the previous non-improved mobile open-circuit respirometry system (268 kJ/kg $BW^{0.75}$ per day; Fernández *et al.*, 2012b) and similar than the obtained in Segureña sheep also by

indirect calorimetry (272 kJ/kg $BW^{0.75}$ per day; Aguilera *et al.*, 1986). Freetly *et al.* (1995, 2002) found no differences for FHP between Suffolk and Texel ewes (318 kJ/kg $BW^{0.75}$ per day, on average) and between Finnsheep and Ramboulliet ewes (297 kJ/kg $BW^{0.75}$ per day, on average) when compared at the same proportion of mature BW, and in accordance with finding of Olthoff *et al.* (1989) with ewes of seven breeds differing in potential of production (296 kJ/kg $BW^{0.75}$ per day, on average). It is well known that differences in FHP could be explained by the previous MEI of the animals. FHP is typically determined after 3 days fast, one might expect less of a difference in energy expenditure by splanchnic tissues than in the fed state. Tovar-Luna *et al.* (2010) determined FHP by lactating dairy goats immediately after ad libitum consumption compared with that following intake near MEm, noting 13% less for the latter condition.

The RQ for fed and fasting sheep were in close agreement with the regular values obtained under such circumstances and could be used as well as an index of reliability of measurements.

Although to compare the diets was not relate with the main objective of the present study, MEI was lower with ALH diet compared with other diets (P < 0.05), however, HP was higher (P < 0.05) for the ALH diet with respect to the other two maybe as result of the different nature and physical structure of the feed consumed since time period spent eating was included in the 24 h gas exchange measurements. Lachica *et al.* (1997) in goats found that the act of eating accounts for an energy cost of about 0.05 of the MEI for long dried forage with similar values between species. In the present experiment represented 23.7 kJ/kg $BW^{0.75}$ per day which corresponded to a theoretical value of 497 kJ/kg $BW^{0.75}$ per day, very close to the obtained of 494 kJ/kg $BW^{0.75}$ per day. This explanation is concordance with the fact that FHP value agreed with the value previously obtained for the same sheep breed by Fernández *et al.* (2012b). As result, the RE was negative for ALH whereas it was positive for CGR and FCH (95 kJ/kg $BW^{0.75}$ per day, on average) diets.

The C-N balance (CN method) is frequently determined in association with indirect calorimetry measurements (Blaxter, 1967) and it depends on measurements of C and N intake and their losses as urine, feces and gases (CO_2 and CH_4). The RE determined with the CN method was greater than the calculated with the RQ method (74 *vs.* 56 kJ/kg $BW^{0.75}$ per day, on average). The CN method generally results in an overestimation of RE because C-N balance is usually overestimated due to evaporative and other losses in excreta (Just *et al.*, 1982). Thus, the RQ method can be expected systematically to yield higher values for HP than the CN method. However, both methods have given no significant different values in each diet, accounting for 431 *vs.* 410, 404 *vs.* 379, and 494 *vs.* 489 kJ/kg $BW^{0.75}$ per day (CGR, FCH and ALH, respectively) for RQ *vs.* CN methods. Both methods are partially dependent of each other, the agreement between them can be another index of the absence of systematic errors. A wide account about the errors attached to the estimation of RE (or HP) by the CN method was given elsewhere (Blaxter, 1967; Christensen *et al.*, 1988). Discrepancies averaged 3.5% when expressed as

percentage of the MEI, a rather satisfactory value taking into account the considerable amount of technical and analytical work involved. Fernández *et al.* (2012b) in sheep using a face mask and the same diet at three different MEI (instead of the same MEI for the three diets in the present study) obtained an average discrepancy of 0.005, and Aguilera and Prieto (1986) in respirometry chambers and fed at about maintenance level of 0.018. As an out-door method, the current mobile open-circuit hood system complies the accuracy requisite for determining gas exchange and, thereafter, the HP (where the maximum acceptable error is 0.1; Lachica and Aguilera, 2008).

CONCLUSIONS

The present study shows the improvements made on a homemade mobile open-circuit respiration system to measure CH_4, CO_2 and O_2 animal exchange and, thereafter, HP. All sheep were easily adapted to the head hood for periods of 24 h with no sing of discomfort or stress. The low-cost system described is suitable and feasible to be adapted and used for studies of gas exchange and energy metabolism under a wide range of physical, physiological and nutritional situations with a considerable labour reduction.

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EXPERIMENT III

Energy partitioning and substrate oxidation by Guirra ewes fed soybean hulls and corn gluten feed blend as a replacement for barley grain

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ABSTRACT

The aim of this experiment was to study the effect of substituting barley grain by soybean hulls and corn gluten feed blend on energy partitioning, substrate oxidation, C-N balance in sheep. The experiment was conducted in a crossover design with 12 dry and non-pregnant adult Guirra ewes of similar body weight (57.5 ± 1.9 kg BW) in each group and sheep were fed two dietary treatments in two 27 d periods. The ewes were fed with two different mixed diets; one group was fed a mixed diet with 365 g barley grain/kg dry matter (DM) (BRL diet) and the other diet (SHCG diet) replaced barley with soybean hulls (271 g/kg DM) and gluten feed (93 g/kg DM). Six sheep per group were used to determine apparent total tract digestibility (10 d adaptation plus 5 digestibility and balance of nutrients), gas exchange (12 d), oxidation of nutrients and C-N balance in each period of the experiment. The average values of metabolizable energy intake, heat production and retention of energy were 498, 436 and 62 kJ/kg $BW^{0.75}$, respectively, with no differences between diets. The estimated value of metabolizable energy for maintenance was 391 kJ/kg $BW^{0.75}$. The efficiency of use of metabolizable energy for maintenance was 0.64 and the nutritive value of the diets was 7.6 MJ of net energy/kg DM, on average. Most of the heat production derived from oxidation of fat (0.48) for the fibrous diet, and from oxidation of carbohydrates (0.63) for the starchy diet, with no differences in total energy retained in tissues (67 kJ/kg $BW^{0.75}$). This fibrous by-product was utilized by the ewes with no detrimental effect on energy metabolism and resulted in a similar positive energy balance to that of a traditional cereal based diet. The economic advantages and sustainability of this choice should be evaluated.

Keywords: Guirra ewes, barley substitution, energy partitioning, substrate oxidation.

INTRODUCTION

The Spanish ruminant production system (Interal, 2008; FEDNA, 2009) is based on high use of concentrate, with mixed diets instead of whole forage rations. By-product feeds have been used extensively in ruminant diets in many parts of the world as economical substitutes for cereals and soybean meal. The partial replacement of cereal grain with low starch by-product feeds represents a potential alternative to reduce the cost of feeding. Soybean hulls and corn gluten feed are typically used as grain replacers. According to NRC (2001), soybean hulls and corn gluten feed are two by-product feeds that are highly digestible but low in non fibrous carbohydrates (NFC); soybean hulls are high in neutral detergent fiber (NDF) with 670 g/kg dry matter (DM) and acid detergent fiber (ADF), but are low in lignin and NFC (140 g/kg DM). Ludden *et al.* (1995) considered soybean hulls to be a bulky concentrate similar to beet pulp rather than roughage, despite the higher fiber content of soybean hulls. According to NRC (2001), corn gluten feed is good source of crude protein (CP, 240 g/kg DM), high in hemicellulose (360 g NDF/kg DM) and moderate in NFC (300 g/kg DM). These by-products are widely available and an affordable source of energy supply for the animal.

Diet composition influences the efficiency of energy metabolism. The respiration quotient method (RQ) was used to determine the associated heat production (HP), and the C-N balance (CN method) was used to calculate the retained energy (RE), assuming that all energy is retained either as fat or protein. The aim of this experiment was to study the effect of substitution of barley grain by soybean hulls and corn gluten feed blend on energy partitioning, substrate oxidation and C-N balance in sheep.

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).

Animals and Feeding

The experiment was conducted with twelve dry and non-pregnant adult Guirra breed ewes kept in two groups and fed 1 of 2 diets in two 27 d periods in crossover design. The twelve ewes had similar body weight (57.5 ± 1.9 kg BW). Ewes were fed with two different mixed diets; one group was fed a mixed diet with 365 g/kg DM of barley grain (BRL diet) and the other diet substituted barley with 364 g/kg DM of by-products (SHCG diet) in the following proportion: 271 soybean hulls and 93 gluten feed, both expressed in g/kg DM. Six ewes per group were used to determine apparent total tract digestibility, gas exchange, oxidation of nutrients and C-N balance; this process was

repeated, crossing the animals. Alfalfa hay was cut into 2.5 cm pieces (Skiold Saby A/S, Kjeldgaardsvej, DK 9300) and the concentrate was mixed and pelleted along with the premix (Table 3.1). Mixed diets contained similar amounts of gross energy (GE, 19.04 MJ/kg DM) and CP (18.0% on DM basis). Fat was included in the SHCG diet to increase the energy content of the fibrous diet (19.4 g/kg DM of lard and 11.7 g/kg DM of by-pass fat of palm FA distillate). Starch levels were 26.35 and 8.32% on DM basis for BRL and SHCG diets, respectively. Nutrient requirements followed the FEDNA recommendation (2009) for sheep. Intake was *ad libitum* with diets offered at 110% of consumption on the preceding few days. Half of the daily ration was offered at 08:00 and another half at 16:00 h, respectively. Ewes had free access to water.

Experimental Schedule and Measurements

Ewes were fed with experimental diets in pens for 10 days and then allocated to individual metabolism cages in thermoneutral conditions (20 to 23 °C determined by a Hobo probe, ONSET data loggers, Cape Cod, MA, USA). After 10 d of adaptation, feed intake, orts and total feces and urine were recorded daily for each ewe over a collection period of 5 d. Body weights of ewes were noted at the beginning and end of the collection 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 1M sulphuric acid to maintain a pH below 5 for later analysis. Samples of hay and concentrate, feces and urine were collected over 5 consecutive days and stored at -20 °C, then pooled for chemical analysis.

Then, gas exchange was measured for each ewe during 24 h (6 sheep/treatment) by a head box designed for small ruminants. To this end, 12 d were taken for each period in the crossover design. The respirometry system has a head hood, a flowmeter (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 CH_4 and CO_2 concentrations were measured using the infrared principle and O_2 concentration was measured by the paramagnetic principle (Easyflow gas analyzer, model 3020, ABB, Alzenau, Germany). Although the unit was an autocalibrated model, the analysers were calibrated with reference gases before each test. Fernández *et al.* (2012a) described the open-circuit respirometry system used for these measurements.

The whole system was calibrated injecting pure N_2 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 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.

Table 3.1. Ingredients and chemical composition of the diets based on barley grain (BRL) or soybean hulls and corn gluten feed blend (SHCG).

	Diets[1]	
	BRL	SHCG
Ingredients (g/kg DM)		
Alfalfa hay	513.2	514.9
Barley	365.0	-
Soybean meal 44%	102.1	79.8
Soybean hulls	-	271.3
Gluten feed 18%	-	92.7
Lard[2]	-	19.4
By-pass fat[3]	-	11.7
Calcium carbonate	8.2	5.7
Bicalcium phosphate	6.3	-
Sodium chloride	2.6	1.9
Premix[4]	2.6	2.6
Chemical composition (% DM)		
Dry matter (%)	87.77	88.75
Organic matter	92.36	91.98
Crude protein	18.55	17.45
Ether extract	2.05	6.25
Neutral detergent fiber	36.80	43.90
Acid detergent fiber	15.03	24.52
Non fibrous carbohydrates[5]	30.85	23.28
Starch	26.35	8.32
Gross energy (MJ/kg DM)	18.92	19.16

[1] BRL = barley diet; SHCG = soybean hulls and gluten feed diet.

[2] Fused lard provided by VALGESS S.L., Carpesa, Valencia, Spain.

[3] By-pass fat of palm fatty acid distillate. Provided by Norel Animal Nutrition, Norel S.A. (Spain).

[4] Provided by NACOOP S.A. (Spain). Premix composition: Se 40 mg/kg; I 250 mg/kg; Co 80 mg/kg; Cu 3,000 mg/kg; Fe 6,000 mg/kg; Zn 23,400 mg/kg; Mn 29,000 mg/kg; S 60,000 mg/kg; Mg 60,000 mg/kg; vitamin A 2,000,000 IU/kg; vitamin D3 400,000 IU/kg; vitamin E 2,000 mg/kg; nicotinic acid 10,000 mg/kg; choline 20,300 mg/kg.

[5] Non fibrous carbohydrates = 100 - (neutral detergent fiber + ash + crude protein + ether extract).

Chemical Analysis

Feed, feed refusal and feces samples were first dried in a forced air oven at 55 °C for 48 h, then ground to pass a 1 mm screen. Urine was dried by lyophilisation. DM of diets, refusal and feces was determined by oven-drying at 102 ± 2 °C for 24 h (no. 934.01, AOAC, 2000). Ash concentration (no. 942.05, AOAC, 2000) was measured by incineration in an electric muffle furnace at 550 °C for 6 h to determine organic matter (OM). Feed offered, orts and feces were analysed for NDF and ADF using the ANKOM fiber analyzer (A220, ANKOM Technologies, Fairport, NY, USA) following procedures of Van Soest *et al.* (1991). The NDF was determined using sodium sulphite and alpha-amylase, both exclusive of residual ash. Ether extract (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) using no. 920.39 of AOAC (2000). Amounts of C and N were analysed by Dumas principle (TruSpec CN; Leco Corporation, St. Joseph, MI, USA) in a total combustion method (no. 968.06, AOAC, 2000). Multiplying N by a factor of 6.25 converted the results to CP. The 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. Starch content was determined by enzymatic method (alpha-amylase obtained from Sigma-Aldrich, Steinheim, Germany) according to Batey (1982). GE content of the dried samples (feed, feces and urine) was analysed by combustion in an adiabatic bomb calorimeter (Gallenkamp Autobomb; Loughborough, UK).

Calculations

Metabolizable energy (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 39.54 kJ/L CH_4; Brouwer, 1965).

Heat production was calculated according to Brouwer (1965) for O_2 consumption, CO_2 and CH_4 production, and urine N (N_{urine}) as:

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

where gases were expressed in L and N_{urine} in g. RE was calculated as the difference between MEI and HP.

Energy associated with the oxidation of protein (OXP), carbohydrate (OXCHO) and fat (OXF) was calculated using the method of Brouwer (1958) and Chwalibog *et al.* (1997) for ruminants:

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

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

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

Then, the HP from oxidation (HPx) was:

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

where gases were expressed in L and N_{urine} in g, and the CO_2 production from oxidation (CO_{2x}) was calculated as $CO_2 - (CO_2/CH_4 \times CH_4)$. Fermentation heat (HPf) was estimated subtracting HP from HPx. The non protein respiratory quotient for oxidation of nutrients (RQnpx) was determined as:

$$\text{RQnpx} = [CO_{2x} - (N_{urine} \times 6.25 \times 0.774)] / [O_2 - (N_{urine} \times 6.25 \times 0.957)]$$

The efficiency (k_m) of use of ME for maintenance (MEm) was calculated considering a fasting heat production (FHP) in Guirra breed ewes of 318 kJ/kg $BW^{0.75}$ (according to López and Fernández, 2013), and k_m = FHP / MEI.

In the CN method, we followed the equation and values proposed by McLean and Tobin (1987). The sum of RE (kJ) in protein ($RE_{protein}$) and fat (RE_{fat}) was RE_{body} and the HP determined by CN method is HP = MEI - RE_{body}.

Statistical Analyses

Data were analysed using the mixed model (proc MIXED) from SAS software (2001). The experiment was conducted as a crossover design; each ewe received both treatments in two periods. Sheep served as the experimental unit for all data. The model for the dependent variables included the fixed effect of diet and period with sheep as random effect. The following statistical model was used; Y = μ + D + T + animal + 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, respectively; animal is the random effect of sheep and ε is the random error. Least square means are reported throughout and differences were considered significant at $P < 0.05$.

RESULTS

No significant effect was observed for the period of time in the crossover design and tables do not show this effect.

Feed intake and digestibility

Total tract apparent digestibility of nutrients for the Guirra ewes is shown in Table 3.2. Dry matter intake was higher ($P < 0.05$) for SHCG than BRL (0.89 *vs.* 0.87 g/d,

respectively). Significant differences (P < 0.05) were observed for digestibility of all nutrients except starch (99.9%, on average). Significantly (P < 0.05) higher values were found in diet BRL than SHCG for DM, OM, CP and GE digestibility, while lower values were found in diet BRL than SHCG for EE and NDF.

Table 3.2. Body weight, dry matter intake and apparent digestibility coefficients of Guirra sheep (n = 24) according to the type of diet.

	Diets[1]		SEM[2]	P-value
	BRL	SHCG		
Body weight (kg)	57.6	57.5	0.79	0.954
Dry matter intake (kg/d)	0.867	0.885	0.001	0.005
Digestibility (%)				
Dry matter	73.0	70.4	0.59	0.018
Organic matter	75.7	72.7	0.62	0.011
Crude protein	80.0	75.1	0.89	0.001
Ether extract	85.7	88.1	0.66	0.032
Neutral detergent fiber	57.4	60.2	0.86	0.043
Starch	99.9	99.8	0.00	0.603
Gross energy	75.9	73.2	0.71	0.044

[1] BRL = barley diet; SHCG = soybean hulls and gluten feed diet.
[2] SEM = standard error of the mean.

Energy partition, oxidation of nutrients and C-N balance

The average value obtained for the calibration factor by releasing a known volume of N_2 into the respirometry system was 1.003 ± 0.01307.

Daily energy balance is shown in Table 3.3. Statistically significant differences (P < 0.05) were observed for GE intake, with greater values for SHCG than BRL (810 *vs.* 788 kJ/kg $BW^{0.75}$, respectively). A tendency (P = 0.099) to greater losses of energy in feces for SHCG diet than BRL was observed (214 *vs.* 192 kJ/kg $BW^{0.75}$, respectively). Urine energy losses and energy losses in CH_4 were not significant between diets (51 and 48 kJ/kg $BW^{0.75}$, on average respectively). The MEI was not different between BRL and SHCG treatments, with an average value of 498 kJ/kg $BW^{0.75}$. The HP and RE were not significant between diets, with an average value of 436 and 62 kJ/kg $BW^{0.75}$, respectively. No significant differences were observed between diets for k_m with an average value of 0.64. The net energy content of the diets was calculated as k_m multiplied by MEI and divided by DMI. No significant differences were found when substituting barley by soybean hulls and gluten feed (the average value obtained was 7.6 MJ net energy/kg DM).

When regressing RE (kJ/kg $BW^{0.75}$) against MEI (kJ/kg $BW^{0.75}$) the efficiency of use of ME obtained for maintenance and positive energy balance was 0.69. The resulting equation from data of the present experiment was RE = -270.4±35.7 + 0.69±0.06 x MEI (R^2 = 0.73; RMSE = 34.67).

Table 3.3. Daily energy balance (kJ/kg $BW^{0.75}$) of Guirra sheep (n = 24) according to the type of diet.

Item[3]	Diets[1]		SEM[2]	P-value
	BRL	SHCG		
Gross energy intake	788.2	810.3	13.81	0.048
Energy in feces	192.4	214.3	7.15	0.099
Energy in urine	55.1	45.9	4.71	0.260
Energy in CH_4	47.0	49.2	3.98	0.643
MEI	493.7	500.9	10.26	0.581
HP	436.5	434.4	6.31	0.568
RE	57.2	66.4	8.53	0.432
k_m	0.64	0.64	0.035	0.978
NE (MJ/kg DM)	7.6	7.6	0.10	0.857

[1] BRL = barley diet; SHCG = soybean hulls and gluten feed diet.
[2] SEM = standard error of the mean.
[3] MEI = metabolizable energy intake; HP = heat production; RE = retained energy, calculated as RE = MEI – HP; k_m = efficiency of metabolizable energy for maintenance assuming a fasting heat production in Guirra breed of 318 kJ/kg $BW^{0.75}$ (López and Fernández, 2013), without correct the MEI; NE = net energy of diet under positive energy balance, calculated as NE = (MEI x k_m) / dry matter intake.

No statistically significant differences were found between the two diets for protein oxidation: OXP was 14% of HPx, on average (Table 3.4). Only a trend in the HP of fermentation was observed (P = 0.067), showing greater fermentation activity on SHCG diet than BRL (15 *vs.* 8 kJ/kg $BW^{0.75}$, respectively), though the amount of methane produced per OM intake was not significant. No significant differences were observed for HPx (424 kJ/kg $BW^{0.75}$, on average). The slight increase in fermentation was accompanied with greater (P < 0.05) values of fat oxidation (OXF increased from 22% to 48% of HPx in BRL and SHCG, respectively) with reciprocal and significant values to OXCHO (from 63% to 38% of HPx in BRL and SHCG, respectively).

Table 3.4. Daily oxidation of protein, carbohydrate and fat (kJ/kg $BW^{0.75}$) and their contribution to the heat production from substrates oxidation (%) of Guirra sheep (n = 24) according to the type of diet.

Item[3]	Diets[1]		SEM[2]	P-value
	BRL	SHCG		
CH_4/OMI (L/kg)	31.1	31.9	1.35	0.875
HPx	429.2	418.9	6.04	0.943
HPf	8.4	15.1	0.04	0.067
OXP	63.9	58.3	2.31	0.400
OXCHO	269.8	159.1	21.79	0.047
OXF	95.4	202.1	19.82	0.014
OXP/HPx	15.2	14.1	6.22	0.421
OXCHO/HPx	62.8	38.3	5.31	0.042
OXF/HPx	22.0	47.6	3.32	0.026
RQnpx	0.91	0.85	0.029	0.197

[1] BRL = barley diet; SHCG = soybean hulls and gluten feed diet.

[2] SEM = standard error of the mean.

[3] CH_4/OMI = methane and organic matter intake ratio; HPx = heat production from oxidation of nutrients; HPf = heat production of fermentation, calculated as HPf = HP – HPx (Brouwer, 1958); OXP = oxidation of protein; OXCHO = oxidation of carbohydrates; OXF = oxidation of fat; RQnpx = non protein respiratory quotient from oxidation of nutrients, calculated as RQnpx = $[CO_{2x} - (N_{urine} \times 6.25 \times 0.774)] / [O_2 - (N_{urine} \times 6.25 \times 0.957)]$, where CO_2 production from oxidation is CO_{2x}.

The daily C-N balance and the calculated energy retained in body tissues, as protein and fat, are displayed in Table 3.5. Significant differences (P < 0.01) were found in C intake, and N intake, with lower C intake values for BRL and greater in N intake. A tendency (P = 0.093) was found in N in feces, with greater values for SHCG than BRL. Significant differences (P < 0.05) were found for RE_{fat} (50 *vs.* 67 kJ/kg $BW^{0.75}$, for BRL and SHCG respectively), although the total RE was positive for the two treatments with no significant differences (67 kJ/kg $BW^{0.75}$, on average).

Table 3.5. Daily C-N balance (g/kg BW$^{0.75}$) and retained energy in body tissues (kJ/kg BW$^{0.75}$) of Guirra sheep (n = 24) according to the type of diet.

Item[3]	Diets[1]		SEM[2]	P-value
	BRL	SHCG		
C balance				
C intake	19.02	19.61	0.347	0.040
C in feces	4.94	5.39	0.146	0.111
C in urine	0.68	0.66	0.025	0.606
C in CO_2	11.32	11.30	0.149	0.754
C in CH_4	0.85	0.88	0.042	0.643
C retained	1.23	1.38	0.244	0.491
N balance				
N intake	1.23	1.18	0.022	0.002
N in feces	0.27	0.30	0.010	0.093
N in urine	0.87	0.84	0.020	0.542
N retained	0.08	0.03	0.032	0.293
RE$_{protein}$	12.8	4.1	4.84	0.293
RE$_{fat}$	49.5	67.2	11.49	0.038
RE$_{body}$	62.3	71.3	12.37	0.407

[1] BRL = barley diet; SHCG = soybean hulls and gluten feed diet.
[2] SEM = standard error of the mean.
[3] RE$_{protein}$ = recovery energy of protein; RE$_{fat}$ = recovery energy of fat;
RE$_{body}$ = RE$_{protein}$ + RE$_{fat}$.

DISCUSSION

Spanish production systems are based on high use of concentrate and less pasture. Alternative feedstuffs (soybean hulls, gluten feed, citrus and beet pulps) are viable replacements for traditional feedstuffs (corn and barley) for ruminants when they are economically priced and available, or when traditional feedstuff prices are high. In this trial we used 490 g/kg DM of concentrate, and 364 g/kg DM of barley grain was replaced by soybean hulls and gluten feed. Thus, the diet with barley had high starch content (26.4%) and the fibrous diet had a low content (8.3%). The partial replacement of barley grain with low starch by-product feeds represents a potential alternative to reduce the cost of the diet, and the main focus of this experiment was to study their influence on energy partitioning and carbon and nitrogen balances.

Feed intake, digestibility and energy partition

Sheep fed the SHCG diet consumed 2% more DM than those in the BRL diet. The higher level of starch and lower level of fiber in BRL diet compared to SHCG appeared to be the main factor responsible for the decrease in DM, OM, CP and GE apparent digestibility in SHCG diet. Fat levels were 2.05 *vs.* 6.25% for BRL diet and SHCG respectively and the digestibility was greater for diet SHCG. The level of fat in BRL diet came from raw source ingredients, and fat was probably linked to carbohydrates and therefore, less available for digestion when compared with the source of external added fat in SHCG diet; lard and by-pass fat. The fiber of by-products has different physical and chemical properties from forage NDF, such as alfalfa hay; in particular, its particles have small dimensions and a high density (Firkins, 1997), although soybean hulls density is light. These characteristics seem to be responsible for the greater NDF digestibility for diet SHCG than for BRL. Starch digestibility was almost complete for the 2 diets. Different authors have reported higher starch apparent digestibility; barley starch is roughly 94% fermented in the rumen, whereas corn starch is around 78% fermented in the rumen of cows and greater for sheep (Waldo, 1973). The BRL diet with higher starch content is accompanied by lower fiber digestibility.

Table 3.3 shows us a daily energy balance, and we found greater GEI for SHCG diet than BRL (3% higher), following the same pattern as DM intake. Ludden *et al.* (1995) reported that the dietary addition of fat at more than 3% DM might decrease digestibility of fibrous feedstuffs, such as soybean hulls, by inhibiting fibrolytic bacteria. In our trial, we observed no decrease in fiber digestibility: around 12 g of fat/kg DM was by-pass, and part of the fat was probably excreted in feces. A 2% DM of fat is relatively low to have a negative effect on fiber digestibility (Patra, 2013). Methane energy values obtained by Hammond *et al.* (2013) with sheep fed 800 g/d of forage (white clover and perennial ryegrass) were 60 kJ/kg $BW^{0.75}$, much greater than in our study, taking into account that this diet was only forage. This seems to indicate that the greater fermentative activity in SHCG diet is probably associated with the forage in the diet (alfalfa hay) and the blend of fibrous by-products (soybean hulls and gluten feed).

Similar values of MEI, HP and RE were found by Fernández *et al.* (2012b) feeding dry and non-pregnant Manchega ewes at maintenance level with a mix of barley and alfalfa hay; 454, 380 and 73 kJ/kg $BW^{0.75}$ for MEI, HP and RE respectively. The value of ME for maintenance, estimated by regression, was 391 kJ/kg $BW^{0.75}$ and, considering this value as maintenance, the feeding level in our trial was estimated as 1.3 times maintenance. Fernández *et al.* (2012b) with Manchega sheep found a value of 383 kJ MEm/kg $BW^{0.75}$ and, López and Fernández (2013) in Guirra sheep found a value of 352 kJ MEm/kg $BW^{0.75}$. Aguilera *et al.* (1986) estimated MEm requirements in Segureña sheep by linear regression at 374 kJ MEm/kg $BW^{0.75}$ per day and the efficiency of utilization of ME for maintenance (k_m) in 0.72. Kearl (1982) from nine reports suggest MEm ranging from 305 to 460 kJ/kg $BW^{0.75}$ per day, and Nsahlai *et al.* (1997) estimated a MEm for Menz sheep of the Ethiopian highlands of 422 kJ/kg $BW^{0.75}$. NRC (2007) and

INRA (2007) adopted an average value (321 and 397 kJ/kg $BW^{0.75}$, respectively) due to the fact that FHP *vs.* feeding trial or, calorimetry studies *vs.* other methods, give different values. AFRC (1998) consider values of MEm ranging from 325 to 378 kJ/kg $BW^{0.75}$, and CSIRO (2007) from 286 to 390 kJ/kg $BW^{0.75}$. Therefore, several factors (confined animals, activity energy cost, intake level, etc.) could contribute to relatively small or high MEm estimates.

Oxidation of nutrients

The CO_2 production is derived from nutrient oxidation and rumen fermentation. So, 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.

With numerical CH_4 production increasing from diet BRL to SHCG (1.19 *vs.* 1.24 L CH_4/kg $BW^{0.75}$, respectively), no significant increase in CH_4 production in relation to OM intake was found; 32 L/kg OM intake, on average (Table 3.4). With increasing fermentation, less glucose was absorbed directly and oxidized as OXCHO, while more carbohydrate was converted to volatile fatty acids and oxidized as OXF. Therefore, most of the HPx derived from OXF (48%) was for SHCG diet and most of OXCHO (63%) for BRL diet. Few studies relating to oxidation of nutrients are available for ruminants and especially for small ruminants. Chwalibog *et al.* (1997), in calves with positive energy recovered as fat, pointed out that part of OXF should originate from ingested carbohydrate, mainly fiber. Fernández *et al.* (2012b) found in Manchega sheep that in fasting conditions most of the HPx was due to OXF (93%), and OXCHO increased up to a stable value of 51% as corn grain consumption increased in the diet.

Although no significant difference was observed for RQnpx, the values were below 1.0, indicating fat oxidation (Chwalibog *et al.*, 1997). There was a tendency of decreasing RQnpx with increasing CH_4 production, and though not significant, the reduction in RQnpx was consistent with the switch between OXCHO and OXF as less carbohydrate and more fat was oxidized, suppressing RQ values.

Nitrogen and carbon balances

In our trial, the ewes were dry and not pregnant and the C efficiency (C retained:C intake) was of 0.06 for both diets. The N efficiency (N retained:N intake) was 0.07 for BRL diet and 0.03 for SHCG diet; so, this lower N efficiency for fibrous diet was linked to less intake and more N excreted in feces. Although fibrous diet showed lower N efficiency, greater energy was recovered as fat, and no differences were observed between diets in total retained energy (67 kJ/kg $BW^{0.75}$, on average). Besides, from C-N balance data is possible to calculate the average daily gain, and the value estimated

was of 68 g/d and 51 g/d for diet BRL and SHCG, respectively. 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). 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 mobilised from body fat tended to be higher in cows fed lipogenic diet *vs.* cows fed the glucogenic diet. So, ewes fed SHCG diet probably obtained more energy from OXF in order to reach the same amount of deposited energy as sheep fed BRL diet, which obtained most of the energy from OXCHO. For dry and non-pregnant ruminants with the same feeding level, it seems that different metabolic pathways were followed to achieve the same energy balance.

CONCLUSIONS

This paper provides data for energy partitioning and substrate oxidation of nutrients in Guirra ewes fed a diet with 365 g barley/kg DM and replaced by the same amount of fibrous by-product (soybean hulls and gluten feed blend). No differences between diets were found for energy balance, so the estimated value of ME for maintenance was 391 kJ/kg $BW^{0.75}$. Therefore, the efficiency of use of ME for maintenance (k_m) was 0.64 and the nutritive value of the diets was 7.6 MJ net energy/kg DM, on average. Different behaviour was found between diets for oxidation of nutrients; the OXF was 0.48 for the by-products diet, and the OXCHO was 0.63 for cereal diet. This fibrous by-product was utilized by the ewes without any detrimental effect on energy metabolism and resulted in a similar positive energy balance to that provided by a traditional cereal based diet. The economic advantages and sustainability of this choice should be evaluated.

ACKNOWLEDGMENTS

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EXPERIMENT IV

Energy partitioning and substrate oxidation by Murciano-Granadina goats during mid lactation fed soybean hulls and corn gluten feed blend as a replacement for corn grain

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ABSTRACT

The aim of this study was to study the effect of substitution of corn grain by soybean hulls and corn gluten feed blend on energy partitioning, substrate oxidation and milk performance in dairy goats during mid lactation. Ten multiparous Murciano-Granadina goats in mid lactation were fed 2 isoenergetic and isoproteic diets [19.08 MJ/kg dry matter (DM) and 18.7% of protein, DM basis] in a crossover design. One group of 5 goats was fed a mixed ration with 373 g corn grain/kg DM (CRN diet) and the other diet replaced corn grain with 373 g fibrous by-products/kg DM [soybean hulls and gluten feed (SHGF) diet]: 227 g soybean hulls/kg DM and 146 g gluten feed blend/kg DM. Fat was added to the SHGF diet to make it isoenergetic. After 10 d of adaptation, the feed intake, refusal, total fecal and urine output, and milk yield were recorded daily over a 5 d period. Then, gas exchange measurements were recorded by a mobile open-circuit respirometry system using a head box for 10 d. Dry matter intake was similar for both diets (2.07 kg/d, on average). Greater and significant values were found in the SHGF diet for ammonia N, energy in urine and oxidation of protein. Values were significantly lower for heat production of fermentation, indicating a decrease in rumen fermentation with this diet, probably due to an excess of crude protein in the diet and lack of synchronization of the non fibrous carbohydrates with rumen-degraded protein. The metabolizable energy intake was no different between CRN and SHGF treatments, with an average value of 1,444 kJ/kg $BW^{0.75}$. Due to the positive energy balance during mid lactation in this trial, most of the heat production from oxidation of nutrients derived from carbohydrate oxidation (55%, on average), followed by oxidation of fat (29%, on average). No significant differences were observed for milk production, although milk fat was significantly greater for SHGF diet than CRN (7.0 *vs.* 5.4%, respectively). Despite the different starch levels and fibrous content used in these mixed diets, no significant differences for the efficiency of use of metabolizable energy for mid lactation were observed (0.63, on average). An average nutritive value of 7.52 MJ net energy of lactation/kg DM was obtained. This fibrous by-product was utilized by lactating goats without detrimental effect on energy metabolism and resulted in similar performance to grain bases diet. The economic advantages and sustainability of this choice should be evaluated.

Keywords: Murciano-Granadina goat, corn substitution, heat production, substrate oxidation.

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. To prevent ruminal upsets and health problems, the NRC (2001) recommended that the maximum NFC in diets for high producing dairy cows should range from 36 to 44%, depending on the total and neutral detergent fiber (NDF) content of forage of the ration. The partial replacement of cereal grain with low starch by-product feeds represents a potential alternative to overcome this limitation.

By-products feeds have been used extensively in dairy cattle diets in many parts of the world as economical substitutes for corn and soybean meal. There is increasing interest in the nutritive value of by-product feeds as nutritionists seek to manipulate NFC concentrations and undegraded intake protein of dairy ruminant diets. Soybean hulls and corn gluten feed are typically used as grain replacers. According to NRC (2001), soybean hulls and corn gluten feed are 2 by-products feeds that are highly digestible but low in NFC; soybean hulls are high in NDF (67%, high in cellulose) and acid detergent fiber (ADF), but are low in lignin and NFC (14%). Ludden *et al.* (1995) considered soybean hulls to be a bulky concentrate similar to beet pulp, rather than roughage, in spite of the higher fiber content of soybean hulls. According to NRC (2001), corn gluten feed is a good source of protein (CP; 24%) much of which is ruminally degraded. Corn gluten feed (36% NDF) is high in hemicellulose and moderate on NFC (30%). Therefore, these by-products are widely available and an affordable source to supply energy for production.

The Spanish production system (Interal, 2008; FEDNA, 2009) is based on high use of concentrate (40 to 70%), with mixed diets instead of whole forage rations. Consuming high levels of concentrate in the diet is common practice in Spain due to the lack of pasture; nutritionists are aware of the importance of ruminants in converting fibrous feeds, unsuitable for direct human consumption, into high quality protein sources such as milk and meat. Goat livestock in Spain occupies the second position after France in the European Union with 30% of the milk production (MAGRAMA, 2012).

The aim of this experiment was to study the effect of substitution of corn grain by soybean hulls and corn gluten feed blend on energy partitioning, substrate oxidation and milk performance in dairy goats during mid lactation.

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).

Animals and Feeding

The experiment was conducted in a crossover design with ten lactating dairy goats kept in 2 groups and fed 1 of 2 diets in two 25 d periods. The ten multiparous mature Murciano-Granadina goats in mid lactation had similar body weight (43.07 ± 2.5 kg BW). Goats were fed 2 different mixed rations; one group was fed a mixed diet with 373 g of corn grain/kg dry matter (DM) (CRN diet) and the other on diet substituted corn with 373 g of by-products/kg DM [soybean hulls and gluten feed (SHGF) diet] in the following proportion: 227 soybean hulls and 146 gluten feed, both expressed in g/kg DM. Five goats per group were used to determine apparent total tract digestibility, gas exchange, oxidation of nutrients and milk yield, in each period of the experiment. Ruminal fermentation parameters were determined in the first period of the experiment. Alfalfa hay was cut into 2.5 cm pieces (Skiold Saby A/S, Kjeldgaardsvej, DK 9300), and the concentrate was mixed and pelleted along with the premix (Table 4.1). Mixed diets were isoenergetic, with an average value of 19.08 MJ/kg DM for gross energy (GE), and isoproteic 18.7% CP (DM basis). Fat was included in the SHGF diet to make it isoenergetic (2.3% lard and 1.3% by-pass fat). Besides, the SHGF diet had 2.3% molasses compared with 0.1% in the CRN diet. Starch levels were 27.99 and 6.57% of DM for the CRN and SHGF diets, respectively. Nutrient requirements followed the recommendation of Lachica and Aguilera (2003) and Fundación Española para el Desarrollo de la Nutrición Animal (FEDNA, 2009) for goats in lactation. Intake was *ad libitum*, with diets offered at 110% of consumption on the preceding few days. Half of the daily ration was offered at 08:00 and half at 16:00 h, respectively. Goats had free access to water.

Experimental Schedule and Measurements

Goats were fed with experimental diets in pens for 10 d and then allocated to individual metabolism cages in thermoneutral conditions (20 to 23 °C determined by a Hobo probe, ONSET data loggers, Cape Cod, MA, USA). After 10 d of adaptation, total tract apparent digestibility, energy balance and ruminal fermentation parameters were determined; the feed intake, refusal and total fecal, urine and milk output were recorded daily for each goat during a 5 d period, as well as the BW at the beginning and end of the period.

Table 4.1. Ingredients and chemical composition of the diets based on corn grain (CRN) or soybean hulls and corn gluten feed blend (SHGF).

	Diets[1]	
	CRN	SHGF
Ingredients (g/kg DM)		
Alfalfa hay	445.9	445.2
Corn	372.8	-
Soybean meal 44%	165.7	113.4
Soybean hulls	-	226.8
Gluten feed 18%	-	145.7
Lard[2]	-	22.7
By-pass fat[3]	-	13.0
Molasses	1.2	23.3
Calcium carbonate	9.7	4.7
Premix[4]	2.6	2.6
Sodium chloride	2.1	2.6
Chemical composition (% DM)		
Dry matter (%)	87.60	88.20
Organic matter	92.51	91.27
Crude Protein	18.60	18.80
Ether extract	2.13	5.04
Neutral detergent fiber	34.82	47.45
Acid detergent fiber	19.06	27.79
Non fibrous carbohydrates[5]	36.96	19.98
Starch	27.99	6.57
Gross energy (MJ/kg DM)	18.76	19.40

[1] CRN = corn diet; SHGF = soybean hulls and gluten feed diet.
[2] Fused lard provided by VALGESS S.L., Carpesa, Valencia, Spain.
[3] By-pass fat of palm fatty acid distillate. Provided by Norel Animal Nutrition, Norel S.A. (Spain).
[4] Provided by NACOOP S.A. (Spain). Premix composition: Se 40 mg/kg; I 250 mg/kg; Co 80 mg/kg; Cu 3,000 mg/kg; Fe 6,000 mg/kg; Zn 23,400 mg/kg; Mn 29,000 mg/kg; S 60,000 mg/kg; Mg 60,000 mg/kg; vitamin A 2,000,000 IU/kg; vitamin D3 400,000 IU/kg; vitamin E 2,000 mg/kg; nicotinic acid 10,000 mg/kg; choline 20,300 mg/kg.
[5] Non fibrous carbohydrates = 100 - (neutral detergent fiber + ash + crude protein + ether extract).

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 of 10% (vol/vol) H_2SO_4 to acidify the urine of each goat. Representative samples (20%) of the diet, feces and urine were collected over 5 consecutive days and stored at -20 °C, and then pooled for chemical analysis. The goats were milked once daily at 08:00 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 weighted and, after mixing, daily samples were 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 apparent digestibility trial. Ruminal fluid pH was immediately determined using a model 265A portable pH meter (Orion Research Inc., Beverly, MA). A ruminal fluid sample was acidified with H_2SO_4 and frozen until later determination of NH_3-N. Samples for analysis of volatile fatty acids (VFA) were mixed with H_3PO_4 and kept frozen until analysis.

Then, gas exchange was measured for each goat for 24 h (5 goats/treatment) using a head box designed for small ruminants. Therefore, 10 d were taken for each period in the crossover design. The respirometry system has 2 separate sampling lines. The main line sucked air through a head box equipped with an air filter to keep dust out. Head hood dimensions were 36 cm deep x 53 cm wide x 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's 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 flowed from the ventilated head hood to the open-circuit respiratory system, which monitored gaseous exchanges by each animal.

The head box was attached to a 2.5 cm (inner diameter) corrugated polyvinyl chloride (PVC) tube connected to the flowmeter. Total air flow through the system was measured by a mass flowmeter with a range from 0 to 10,000 L/h (thermal mass flowmeter Sensyflow VTS, ABB, Alzenau, Germany) and regulated by a manual valve at 3,000 L/h. Air suction was by a centrifugal fan (CST60 Soler & Palau Inc., Parets del Vallès, Barcelona, Spain) with 310 m^3/h as maximum capacity located at the end of the main sampling line, with free escape for the air. Total flow was recorded (totalizer VT-S, ABB, Alzenau, Germany) and screen (C150 Universal Process Indicator, ABB, Alzenau, Germany) displayed. In addition, the mass flowmeter had a digital output and was connected to a computer. A subsampling line (polyethylene tubing, 5 mm inner diameter) was located after the mass flowmeter to take a sample of gas from the main line using a membrane pump (ABB, Alzenau, Germany) of 250 L/h capacities. It was attached to a rotameter (DK800, ABB, Alzenau, Germany) with a valve to set the desired flow rate into the gas analyser (Easyflow 3020 model, ABB, Alzenau, Germany). The CH_4

and CO_2 were measured by using the infrared principle with a concentration range from 0 to 0.15 and 0 to 1.5%, respectively; O_2 was measured by using the paramagnetic principle from 19 to 21% and equipped with an atmospheric compensation module to offset changes in pressure. Although the unit was an autocalibrated model, before each test, analysers were calibrated with reference gases. This analyzer was controlled by Modbus communication protocol with a personal computer (Fujitsu Siemens Lifebook Series, Pentium 4 laptop, Munich, Germany) under a LabVIEW software (http://www.ni.com/labview) environment to save the data to the hard disk each minute. Fernández *et al.* (2012a) describes the mobile open-circuit respirometry system used for these measurements.

The whole system was calibrated by injecting pure N_2 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 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.

Chemical Analysis

Feed, feed refusal and feces samples were first dried in a forced air oven at 55 °C for 48 h then ground to pass a 1 mm screen. Urine was dried by lyophilization. Chemical analyses of the diet, refusals and feces were conducted for DM, ash, ether extract (EE) and CP according to AOAC (2000). The DM content of diets and feces was determined by oven drying at 102 ± 2 °C for 24 h and OM content 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 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 alpha-amylase. The NFC content of diets was calculated by the 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 in an adiabatic bomb calorimeter (Gallenkamp Autobomb; Loughborough, UK). Milk composition (fat, protein, lactose, and total milk solids content) was analyzed with infrared analyzer (MilkoScan FT120 Foss Electric A/S, Hillerød, Denmark). The starch content was determined by the enzymatic method (alpha-amylase obtained from Sigma-Aldrich, Steinheim, Germany) according to Batey (1982). The 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.

Calculations

Metabolizable energy (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 39.54 kJ/L CH_4; Brouwer, 1965). Heat production (HP) was calculated according to Brouwer (1965) for O_2 consumption, CO_2 and CH_4 production, and urine N (N_{urine}) as follows:

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

where gases were expressed in L and N_{urine} in g. The non protein respiratory quotient (RQnp) was determined as: $RQnp = [CO_2 - (N_{urine} \times 6.25 \times 0.774)] / [O_2 - (N_{urine} \times 6.25 \times 0.957)]$. Retained energy (RE) was calculated as the difference between MEI and HP. The retained energy in the body (RE_{body}) was the difference between RE and milk energy (E_{milk}).

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 production of VFA from carbohydrate fermentation is followed by CO_2 and CH_4 production. Fahey and Berger (1988) demonstrated a ratio CO_2:CH_4 of 3:1 and 1.7:1 for high-grain and high-forage diets, respectively. To estimate the CO_2:CH_4 ratio of our diets, VFA was determined and as diets contained 45% alfalfa hay, a value of this ratio was obtained. The CO_2 production from oxidation (CO_{2x}) was calculated as $CO_2 - (CO_2/CH_4 \times CH_4)$. The calculations were carried out as follows:

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

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

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

Then, the HP from oxidation (HPx) was calculated as follows:

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

where gases were expressed in L and N_{urine} in g. Heat of fermentation was estimated subtracting HP from HPx.

The efficiency of use of ME for lactation in absence of change in body energy stores was calculated according to the Agricultural Research Council (ARC, 1980). 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-ARC}) was calculated as corrected milk energy/(ME - MEm), being MEm the metabolizable energy for maintenance, which was obtained from the estimation of Aguilera *et al.* (1990) for Granadina goats from both positive and negative energy retentions (401 kJ/kg $BW^{0.75}$).

As ME could be used for milk synthesis and energy storage in the body, an attempt to estimate the efficiency of ME for milk and body retention is proposed. Recovery energy was partitioned into 2 portions; one used for milk production (E_{milk}) and the other for energy gain in the body tissues (RE_{body}). The basic relationship was expressed quantitatively in the following set of equations:

$$k_l = E_{milk} / (Prop \times MEA)$$

$$k_g = RE_{body} / (1 - Prop) \times MEA$$

where k_l is the efficiency of use of ME for milk production and k_g is the efficiency of ME for tissue gain. The proportion Prop represents the fraction of the ME available for productive purposes (MEA) that is used for milk synthesis and (1 – Prop) is the fraction of MEA used for body tissue energy gain; MEA was calculated by subtracting maintenance requirements from MEI, where maintenance was obtained from estimations (401 kJ/kg $BW^{0.75}$) by Aguilera *et al.* (1990). The next Michaelis-Menten equation was assumed to express the effect of MEA on the partition coefficient:

$$Prop = Prop_{max} \times MEA / (Z + MEA)$$

where $Prop_{max}$ is the maximum fraction of MEA that can be used for milk production and Z represents the level of MEA at which 50% of maximum fraction of MEA is used for milk production.

Statistical Analyses

The effects of corn substitution with by-products on intake, digestibility, ruminal fermentation parameters, milk yield, energy balance and oxidation of nutrients 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. Goat served as the experimental unit for all data. 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: $Y = \mu + D + T + goat + \varepsilon$, where Y is the dependent variable, μ is the overall mean, D and T are the fixed effects of diet and period of time, respectively; goat is the random effect of goat; and ε is the random

error. Least squares means are reported throughout and differences were considered significant at P < 0.05.

RESULTS AND DISCUSSION

No significant effect was observed for the period of time in the crossover design and tables do not show this effect.

Feed Intake, Digestibility and Rumen Fermentation

Spanish production systems are based on high use of concentrate and less pasture. Our study shows a representative diet (55% concentrate) for high production dairy goats. Alternative feedstuffs (soybean hulls, gluten feed, citrus pulp and beet pulp) are viable replacements for traditional feedstuffs (corn and barley) for lactating ruminants when they are priced economically and available, or when traditional feedstuff prices are high. Corn is mainly imported from other countries and so is very costly today in Spain. In this trial, considering the importance of ruminants in converting fibrous feeds into protein, corn was partially substituted with fibrous by-products. The CRN diet had an NFC level of 37% and NDF of 35%, values almost within the range recommended by NRC (2001) for dairy cows (NFC maximum is between 25 and 33%). However, the SHGF diet had a NFC value of 20% and NDF of 47% due to the substitution of corn, mainly with fibrous by-products.

The partial replacement of corn grain with low-starch by-products, such as soybean hulls, represent a potential alternative to overcome this limitation. In this study, the NFC was 37% and 20% of DM in CRN and SHGF, respectively (Table 4.1).

Intake and total tract apparent digestibility of nutrients by Murciano-Granadina dairy goats are shown in Table 4.2. The DMI was similar for both diets (2.07 kg/d, on average). Total apparent tract digestibility was significant (P < 0.05) in DM, OM, EE and NDF. The numerically higher level of starch and lower level of fiber in CRN diet compared with the SHGF diet appeared to be the main factor responsible for the decrease in DM and OM apparent digestibility in the SHGF diet; that is to say, significant reduction in DM and OM digestibility with increasing NDF content (from 35 to 48% NDF on DM basis for CRN and SHGF, respectively) and ADF content (from 19 to 28% ADF on DM basis for CRN and SHGF, respectively) combined with the greater value of starch for CRN diet than SHGF diet (30 *vs.* 7% starch, DM basis). Similar tendencies were observed in lactating dairy goats when the level of ADF increased from 14 to 26% (Santini *et al.*, 1992).

Table 4.2. Body weight, dry matter intake and apparent digestibility coefficients of Murciano-Granadina goats (n = 20) during mid lactation according to the type of diet.

	Diets[1]		SEM[2]	P-value
	CRN	SHGF		
Body weight (kg)	43.08	43.05	0.194	0.954
Dry matter intake (kg/d)	2.04	2.10	0.027	0.067
Digestibility (%)				
Dry matter	67.6	63.8	0.83	0.041
Organic matter	69.2	65.1	0.86	0.029
Crude protein	69.2	70.6	0.75	0.440
Ether extract	66.4	83.9	2.37	0.001
Neutral detergent fiber	36.6	49.5	1.89	0.001
Starch	99.1	99.6	0.09	0.567
Gross energy	68.8	66.2	0.91	0.265

[1] CRN = corn diet; SHGF = soybean hulls and gluten feed diet.
[2] SEM = standard error of the mean.

Fats are commonly added to diets for dairy animals to increase energy density and improve handling characteristics. Greater (P < 0.05) digestibility of EE was found for the SHGF diet than the CRN diet. Although the levels were 2 *vs.* 5% EE for the CRN and SHGF diets, respectively, the level of fat in the CRN diet came from raw source ingredients, and fat was probably linked to carbohydrates and, therefore, less available for digestion compared with the source of external added fat for the SHGF diet: lard and by-pass fat.

NDF digestibility was significant greater for the fibrous diet (50 *vs.* 37% for the SHGF and CRN diets, respectively), probably due to the source of fibrous ingredients (soybean hulls, gluten feed and alfalfa hay) with different physical and chemical properties, compared with the CRN diet, where the main supply of fiber came from alfalfa hay. The fiber of by-products has different physical and chemical properties from forage NDF; in particular, its particles have small dimensions and a high density (Firkins, 1997), although soybean hulls density is light. Starch digestibility was almost complete for the 2 diets, although the SHGF diet had a lower amount of starch (7 *vs.* 28% starch, DM basis, respectively). Different authors reported high starch apparent digestibility; barley starch is roughly 94% fermented in the rumen, whereas corn starch is around 78% fermented in the rumen of cows and greater for sheep (Waldo, 1973). A review by Huntington (1997) of ruminant starch utilization reported ruminal digestibility of whole corn to be 60% and total tract digestibility as 92%. Therefore, this latter value is affected by the corn source, ruminant species, processing method and percentage of corn in the ration. The CRN diet with higher starch content is accompanied by lower fiber digestibility.

Rumen fermentation parameters obtained are shown in Table 4.3. No effect of period of time is shown because rumen liquid extraction was done only in the first part of the trial. The average rumen pH never fell below 6.2, so the values obtained can be considered sufficiently high to maintain normal rumen fermentation. The highest fibrous ingredients in SHGF diet were the cause of the greater pH (P < 0.05) than in CRN diet. Goats fed fibrous by-products had greater ammonia N production (P < 0.05) than those fed the CRN diet (49.58 *vs.* 31.77 mg/dL, respectively), probably linked to the lower N expenditure by ruminal bacteria to synthesize microbial protein (Casper *et al.*, 1999). These greater values obtained are likely due to the average value of CP in diets (18.7% CP, DM basis) and because of N recycling, which appears more extensive in goats than cattle and sheep (NRC, 2007). Bava *et al.* (2001) with Saanen dairy 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.

Table 4.3. pH, ammonia N (NH_3-N) and volatile fatty acids of Murciano-Granadina goats (n = 20) during mid lactation according to the type of diet.

	Diets[1]		SEM[2]	P-value
	CRN	SHGF		
pH	6.86	6.90	0.011	0.048
NH_3-N (mg/dL)	31.77	49.58	4.458	0.048
VFA[3] (mol/100 mol)				
Acetic acid	60.26	64.22	0.786	0.007
Propionic acid	19.98	17.15	1.030	0.176
Butyric acid	11.93	11.61	0.254	0.538
Isobutyric acid	2.04	1.93	0.166	0.759
Isovaleric acid	3.51	3.01	0.255	0.340
η-valeric acid	1.97	1.79	0.056	0.095
η-caproic acid	0.30	0.30	0.025	0.990

[1] CRN = corn diet; SHGF = soybean hulls and gluten feed diet.
[2] SEM = standard error of the mean.
[3] VFA = volatile fatty acids.

The ratios acetic to propionic acids were greater than 3.0 for both diets, possibly because the end products of fiber fermentation, due to cellulolytic organism activity, include a high proportion of acetic acid (both diets had 45% alfalfa hay). A significant increase of acetic acid in the rumen when goats fed the more fibrous diet was observed (60.3 and 64.2 mol/100 mol for CRN and SHGF diets, respectively). The rest of the VFA showed no significant differences between diets. So, SHGF diet had low starch content and the energy it provides would be mainly in the form of highly digestible fiber, as Drewnoski *et al.* (2011) found in growing steers when supplement hay with soybean hulls and corn gluten feed.

Energy Balance

The average value obtained for the calibration factor by releasing a known volume of N_2 into the respirometry system was 1.003 ± 0.01307. The daily energy balance is displayed in Table 4.4. Statistically significant differences (P < 0.05) were observed for GE intake. Energy losses in feces were significant (P < 0.05) and greater for the SHGF diet than CRN diet, probably due to the higher level of fat in the diet. Ludden *et al.* (1995) reported that the dietary addition of fat at more than 3% DM may decrease digestibility of fibrous feedstuffs such as soybean hulls by inhibiting fibrolytic bacteria. In our trial, we observed no decrease in fiber digestibility, probably due to the excretion of fat in feces. Dietary differences in urine energy losses of the SHGF diet were significant (P < 0.05) and higher than in the CRN diet (46.9 *vs.* 23.4 kJ/kg $BW^{0.75}$, respectively) due to lack of efficiency for protein use (greater values of ammonia N on ruminal liquor; Table 4.3) and probably high level of CP in the diets; 18.7% on DM basis, on average). Ruminal ammonia N not used for microbial protein synthesis is likely to be excreted in urine (Hoover and Stokes, 1991). Islam *et al.* (2000) found similar results where the fibrous diet was also higher in energy losses in urine (43.7 kJ/kg $BW^{0.75}$) than less fibrous diet (29.1 kJ/kg $BW^{0.75}$). Bava *et al.* (2001) found values of 62 and 99 kJ/kg $BW^{0.75}$ for diets with 14.8 and 21.4% CP (on DM basis), respectively.

The greater digestibility of DM and OM, lower ammonia N and greater heat of fermentation would seem to indicate that more fermentative activity was taking place with the CRN diet. The HP of fermentation was significantly different (P < 0.05), showing greater fermentation activity in the CRN than the SHGF diet (16.03 *vs.* 14.30 kJ/kg $BW^{0.75}$, respectively). Therefore, energy losses in CH_4 were greater for CRN diet (101.38 kJ/kg $BW^{0.75}$) than SHGF diet (96.44 kJ/kg $BW^{0.75}$), although not significant (P = 0.069). Methane energy values obtained by other authors for lactating goats were within the range obtained in this experiment, so Aguilera *et al.* (1990) found values from 83 to 117 kJ/kg $BW^{0.75}$, both with diets based on pelleted alfalfa hay and barley. Bava *et al.* (2001) did not observe significant differences, but the value of energy losses as CH_4 was 131 and 115 kJ/kg $BW^{0.75}$ for Saanen goats at mid lactation fed a silage-based forage diet and a non forage diet, respectively. In that study, the ammonia N followed opposite trend to that in our trial: 22.8 *vs.* 53.3 mg/dL for forage and non forage diets, respectively. It is generally accepted that utilization of ammonia N for rumen microbial protein synthesis when diets contain a greater amount of NFC. The major factor limiting utilization of N was a readily available energy source. In our study, the CRN diet may have been fermented more readily in the rumen than SHGF diet. Also at mid lactation, Tovar-Luna *et al.* (2010) with Alpine goats reported an average value of 85 kJ CH_4/kg $BW^{0.75}$ when does consumed 60% concentrate diet (31% NDF), and 63 kJ/kg $BW^{0.75}$ for does consuming 20% concentrate (43% NDF), although those authors did not show any ruminal parameter or provide information about the starch level. In our study, it seems that greater dietary concentration of starch increased the utilization of ruminal ammonia N for synthesis of microbial protein, as reported Hoover and Stokes (1991).

Table 4.4. Daily energy balance (kJ/kg $BW^{0.75}$) of Murciano-Granadina goats (n = 20) during mid lactation according to the type of diet.

Item[3]	Diets[1]		SEM[2]	P-value
	CRN	SHGF		
GEI	2285.8	2427.2	40.58	0.001
Energy in feces	725.0	831.3	33.46	0.047
DEI	1560.8	1595.9	14.67	0.164
Energy in urine	23.4	46.9	4.54	0.005
Energy in CH_4	101.4	96.4	1.41	0.069
MEI	1435.9	1452.6	13.88	0.540
HP	801.1	788.7	8.62	0.076
HPf	16.0	14.3	0.28	0.001
RE	634.8	663.9	13.05	0.270
E_{milk}	531.8	542.4	21.95	0.093
RE_{body}	103.0	121.4	25.84	0.753
$k_{l\text{-}ARC}$	0.62	0.64	0.008	0.226
k_l	0.72	0.71	0.007	0.360
k_g	0.35	0.42	0.007	0.754
RQnp	1.03	1.03	0.023	0.386
NE_L (MJ/kg DM)	7.44	7.59	0.095	0.557

[1] CRN = corn diet; SHGF = soybean hulls and gluten feed diet.
[2] SEM = standard error of the mean.
[3] GEI = gross energy intake; DEI = digestible energy intake; MEI = metabolizable energy intake; HP = heat production; HPf = heat production of fermentation [HPf = HP – HP of oxidation (Brouwer, 1958)]; RE = retained energy (RE = MEI – HP); E_{milk} = retained energy in milk; RE_{body} = retained energy in body tissues (RE_{body} = MEI - HP - E_{milk}); $k_{l\text{-}ARC}$ = efficiency of use of ME for milk production [$k_{l\text{-}ARC}$ = corrected milk energy / (ME - MEm), ARC (1980)]; k_l = efficiency of ME for milk production (k_l = E_{milk} / P x MEA and MEA = MEI – 401); k_g = efficiency of ME for tissue retention [k_g = RE_{body} / (1 - P) x MEA, where (1 – P) = the fraction of MEA used for body tissue gain]; RQnp = non protein respiratory quotient {RQnp = [CO_2 – (N_{urine} x 6.25 x 0.774)] / [O_2 – (N_{urine} x 6.25 x 0.957)], where N_{urine} = urine N}; NE_L = net energy of lactation [NE_L = (MEI x $k_{l\text{-}ARC}$) / DMI].

The MEI was no different between the CRN and SHGF treatments, with an average value of 1,444 kJ/kg $BW^{0.75}$. The HP was not significant between diets, with an average value of 794 kJ/kg $BW^{0.75}$, although a tendency to higher values in the CRN diet was observed (P = 0.076). This value is in the range of some values in the literature: Bava *et al.* (2001) found average values of 855 and 637 kJ/kg $BW^{0.75}$ for Saanen goats at mid and late lactation, respectively, and Tovar-Luna *et al.* (2010) with Alpine goats at mid lactation, reported an average value of 737 kJ/kg $BW^{0.75}$ when does consumed a 60% concentrate diet, and 634 kJ/kg $BW^{0.75}$ for does consuming 20% concentrate. No significant differences were observed for E_{milk} content (537 kJ/kg $BW^{0.75}$, on average),

whereas a tendency (P = 0.093) to a greater value for the SHGF diet than the CRN diet was found, probably linked to the significantly higher milk fat content value in the SHGF diet (Table 4.6). Therefore, the energy retained in the body tissues did not differ between the CRN and SHGF diets (112 kJ/kg $BW^{0.75}$, on average).

The efficiency of use of ME for milk production according to ARC (1980; k_{l-ARC}) was calculated as E_{milk} output adjusted to zero energy balance divided by 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 $BW^{0.75}$). No significant differences were observed between diets for k_{l-ARC} (0.63, 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. Those authors found variability when combining different stages of lactation with feed intake level, one of the reasons for differences in efficiencies being related to the complexity of biochemical transformation in tissues that are being synthesized or mobilized.

Dairy animals mobilize energy from body tissue to support energy requirements for milk production during early lactation and replenish mobilized tissue reserves during mid and late lactation for the subsequent lactation. It is generally accepted that the utilization of ME for milk secretion (k_l) in lactating cows in positive energy balance is a more efficient process than that for tissue retention (k_g) by non lactating cows (ARC, 1980). However, doubt exists concerning the efficiency with which energy is retained in tissue when it occurs simultaneously with lactation. In this study, we found no significant differences between diets and greater efficiency of ME use for lactation than retention. In our attempt to estimate the efficiency of ME for milk and body retention, we found an average value of 0.72 for k_l and 0.39 for k_g. Yan *et al.* (1997) indicated that a decrease in energy of 1 MJ/d for milk synthesis was associated with an increase in tissue energy retention of 0.96. Those authors recommend for practical purposes the same value for k_l and k_g. Moe *et al.* (1970) found that the efficiency of energy deposition in lactating cows was as high as the efficiency of use for milk production. This is the view of Ministry of Agriculture, Fisheries and Food (MAFF, 1984), which used the value 0.62 for both ME conversion efficiencies. As mentioned above, the ARC (1980) predicts k_g for energy storage during lactation as $0.95k_l$. The Commonwealth Scientific and Industrial Research Organisation (CSIRO, 2007) reported a general value of 0.60 for conversion of ME to gain; the same value for k_l and k_g. The evidence in the literature in comparative efficiencies of ME utilization for milk secretion and for concomitant tissue retention is conflicting. Higher k_l than k_g may partially relate to the relatively lower mass of tissue contributing to RE that must be maintained by lactating than growing/finishing animals, thereby resulting in a relatively lower energy turnover:synthesis ratio for lactation (NRC, 2007). Furthermore, the contribution of lactose to energy in milk and relatively less energy in protein in milk than in accreted tissue by growing animals in many instances may be involved in high k_l relative to k_g.

The net energy content of the diets was calculated (NE_L, MJ/kg DM) as $k_{l\text{-}ARC}$ (adjusted to zero energy balance) multiplied by ME. No significant differences were found when substituting corn by soybean hulls and gluten feed and the feeding level was 3.6 times maintenance [assuming 401 kJ MEm/kg $BW^{0.75}$ according to Aguilera *et al.* (1990)]. The average value obtained was 7.52 MJ/kg DM. Bava *et al.* (2001) with goats at mid lactation found values of 8.53 and 7.70 MJ/kg DM for silage basis diet and non forage diet, respectively.

Oxidation of Nutrients

The CO_2 production is derived from nutrient oxidation and rumen fermentation. So, separation between these 2 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 production of volatile fatty acids (VFA) from carbohydrate fermentation is followed by CO_2 and CH_4 production. From the molar proportion of VFA (Table 4.3) it is possible to obtain an estimation of the CO_2:CH_4 ratio, following the theoretical rumen fermentation balance proposed by Wolin (1960): 1.7 and 1.6 for diet the CRN and SHGF diets, respectively. The proportional contribution to HPx due to oxidation of nutrients with the 2 diets is shown in Table 4.5.

Table 4.5. Daily oxidation of protein, carbohydrate and fat (kJ/kg $BW^{0.75}$) and their contribution to the heat production from substrates oxidation (%) of Murciano-Granadina goats (n = 20) during mid lactation according to the type of diet.

Item[3]	Diets[1]		SEM[2]	P-value
	CRN	SHGF		
HPx	785.1	774.4	8.47	0.110
OXP	104.3	137.7	7.42	0.001
OXCHO	447.7	412.0	51.05	0.272
OXF	232.9	224.3	49.84	0.557
OXP/HPx	13.3	17.8	0.98	0.001
OXCHO/HPx	56.9	53.5	6.59	0.281
OXF/HPx	29.7	28.7	6.59	0.499

[1] CRN = corn diet; SHGF = soybean hulls and gluten feed diet.
[2] SEM = standard error of the mean.
[3] HPx = heat production from oxidation of nutrients; OXP = oxidation of protein; OXCHO = oxidation of carbohydrates; OXF = oxidation of fat.

Oxidation of nutrients was not statistically different between the 2 diets, with the exception of protein. First, no significant differences were observed for HPx (780 kJ/kg $BW^{0.75}$, on average). The OXP was significantly (P < 0.05) greater for the SHGF than the

CRN diet (138 *vs.* 104 kJ/kg BW$^{0.75}$, on average). The heat from OXP contributed approximately 13% and 18% of HPx in the CRN and SHGF diets, respectively (P < 0.05). As previously mentioned, the SHGF diet decreased the utilization of ammonia N (Table 4.3). This probably accounts for the greater values of OXP observed for the SHGF diet; an asynchrony seems to exist between rumen carbohydrate degradability and rumen degraded protein to maximize microbial protein synthesis to support milk production, as reported by Casper *et al.* (1999).

Due to the positive energy balance, without significant differences between diets during mid lactation in this trial, the OXCHO (430 kJ/kg BW$^{0.75}$, on average) was higher than OXF (229 kJ/kg BW$^{0.75}$, on average). Taking average figures, most of the HPx derived from OXCHO (55%, on average), followed by OXF (29%, on average). It seems that the greater OXCHO and lower OXF found in the 2 diets would be, in part, responsible for the positive energy retained in the body tissues (112 kJ/kg BW$^{0.75}$, on average; Table 4.4). When carbohydrate-rich diets were offered, van den Borne *et al.* (2007) report that animals tended to have increased rates of de novo fatty acid synthesis from glucose with low feed frequency and less fat mobilization. In our study, fibrous by-products had similar behaviour to the diet rich in corn (high starch level). We must bear in mind that the gas exchange method does not discriminate between oxidation of exogenous and endogenous glucose, so this more closely represents net catabolism of glucose. Few studies relating to oxidation of nutrients are available for ruminants and especially for small ruminants. Chwalibog *et al.* (1997) in calves with positive RE as fat pointed out that part of OXF should originate from ingested carbohydrate, mainly fiber. Fernández *et al.* (2012b) found in Manchega sheep that in fasting conditions most of the HPx was due to OXF (93%), although OXCHO increased with a feeding level up to a stable value of 51%.

Milk Production

As the diets were balanced in nutrient requirements for lactating goats and both were mixed diets, goats had similar and non significant milk yield, despite the different amount of starch (Table 4.6). Average milk yield was 2.18 kg/d, and this is consistent with the results obtained by Sanz Sampelayo *et al.* (1998) in Granadina goats fed long or pelleted alfalfa hay and with those of Bava *et al.* (2001) with Saanen goats or Tovar-Luna *et al.* (2010) with Alpine goats.

Milk fat content was statistically different between the 2 diets (7.02 and 5.44% for the SHGF and CRN diets, respectively) and consequently milk DM content (16.60 *vs.* 15.01%). This is because the SHGF diet had higher fiber content than the CRN diet (48 *vs.* 35% NDF, on average), greater (P < 0.05) NDF digestibility than the CRN diet (50 *vs.* 37%, respectively), and higher (P < 0.05) acetic acid production (64 *vs.* 60 mol/100 mol, respectively). Van Knegsel *et al.* (2007) showed that the milk fat level was usually elevated after feeding extra lipogenic nutrients; these originated from fiber, or from

dietary fat or derived from body reserves. Those authors replaced 27% of corn with 29% of beet pulp in dairy cows. Mahjoubi *et al.* (2009), adding beet pulp to dairy cows at late lactation in substitution of barley grain, increased milk energy output mainly because of increased milk fat output. Milk protein and lactose contents were not statistically different between treatments (4.06 and 4.73% respectively, on average).

Table 4.6. Daily milk production and composition of Murciano-Granadina goats (n = 20) during mid lactation according to the type of diet.

	Diets[1]		SEM[2]	P-value
	CRN	SHGF		
Milk production (kg/d)	2.24	2.11	0.079	0.365
Milk composition (%)				
Dry matter	15.01	16.60	0.306	0.002
Fat	5.44	7.02	0.558	0.029
Protein	4.04	4.07	0.091	0.529
Lactose	4.74	4.71	0.062	0.833

[1] CRN = corn diet; SHGF = soybean hulls and gluten feed diet.
[2] SEM = standard error of the mean.

CONCLUSIONS

This paper provides data for energy partitioning and substrate oxidation of nutrients in Murciano-Granadina goats during mid lactation fed a diet with 37% corn and substituted by the same amount of fibrous by-product (soybean hulls and gluten feed blend). The MEI was not different between cereal and by-products treatments, with an average value of 1,444 kJ/kg BW$^{0.75}$. The efficiency of use of ME for milk production (k_{l-ARC}) adjusted to zero energy balance was 0.63. The nutritive value of the diets was 7.52 MJ NE$_L$/kg DM, on average. No significant differences between diets were found for HPx derived from OXF (29%, on average) and from OXCHO (55%, on average). Greater and significant values were found for fibrous by-products diet for ammonia N, urine energy losses, OXP, and significantly lower for HP of fermentation, indicating a decrease in rumen fermentation for this diet. Probably due to an excess of CP in the diet and lack of synchronization of the NFC and rumen degraded protein. Milk production and DMI were similar for goats fed diet based on corn than those for goats fed diet based on fibrous by-products. Milk fat and acetic acid were greater for goats fed soybean hulls and corn gluten feed blend as a replacement for corn grain. This fibrous by-product was utilized by lactating goats without detrimental effect on energy metabolism and resulted in similar performance to a traditional diet. The economic advantages and sustainability of this choice should be evaluated.

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EXPERIMENT V

Use of dry citrus pulp or soybean hulls as a replacement for corn grain in energy and nitrogen partitioning, methane emissions and milk performance in lactating Murciano-Granadina goats

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ABSTRACT

The aim of this study was to assess the effect of substitution of dietary corn grain by dry citrus pulp or soybean hulls on energy and nitrogen partitioning, substrate oxidation, methane emission, and milk performance in dairy goats during mid lactation. Twelve multiparous Murciano-Granadina goats of similar body weight (41.7 ± 2.8 kg of BW) were split in 3 groups in an incomplete crossover design. One group of 4 goats was fed a mixed ration with 605 g/kg of dry matter of corn grain (CRG), another group replaced corn grain with dry citrus pulp (CTP) and the last with soybean hulls (SYH). 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 d 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 3 groups (1.53 kg/d, on average). Total replacement of the concentrate with fibrous by-products increased fiber apparent digestibility. The metabolizable energy intake was significantly greater for diet CRG than SYH (1,193 *vs.* 1,079 kJ/kg $BW^{0.75}$, respectively), CTP showed an intermediate value. The heat production was higher for the fiber diet than starchy diet (908 *vs.* 843 kJ/kg $BW^{0.75}$ for SYH and CRG, respectively). The efficiency of use of metabolizable energy for milk production obtained by regression was 0.59. Goats fed CTP and SYH diets produced similar CH_4 emissions (34.8 g/d, on average), significantly higher compared to goats fed the CRG diet (24.7 g/d). Goats of the 3 treatments were in negative energy balance, so the oxidation of fat was greater than for carbohydrates. No significant differences were observed for milk production (1.72 kg/d), and milk fat was significantly greater for a more fibrous diet compared with a starchy diet (6.57 *vs.* 4.95% in SYH and CRG, respectively).

Keywords: lactating goat, cereal replacement, by-product, energy partitioning, methane emission.

INTRODUCTION

In Mediterranean countries, ruminant production is limited by the poor quality and scarcity of pastures, especially during periods of drought. Consequently, farmers are forced to use cereal-based concentrates and the demand of cereals for animal feeding increases the price of concentrate. This justifies the need to develop feeding strategies based on cost-effective alternatives and local feed resources, which would increase the sustainability of livestock production systems and improves farmers' income. Agricultural by-products may be of interest not only to reduce feed cost, but also to reduce environmental problems associated with by-product accumulation (Vasta *et al.*, 2008).

Increasing interest in by-products has occurred as partial substitution of traditional feedstuffs in ruminant feeding has become more prevalent. 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 with citrus pulp or soybean hulls, which are typically used as grain replacers. A large number of the citrus by-products feedstuffs, including citrus 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 level of dry citrus pulp is intermediate (25% NDF) between that of most concentrates and forages, contains relatively large amounts of pectins (25%) and sugars (23%), a small amount of lignin (2%), and a very limited amount of available nitrogen (6% crude protein, CP). Soybean hulls is high in NDF (58%, high in cellulose) but low in lignin (2%) and non fibrous carbohydrates (24% NFC).

Dietary composition affects the ruminal fermentation and, consequently, energy metabolism, CH_4 emissions and milk composition. CH_4 is an undesirable end product of rumen fermentation as it represents an energy loss to the animal and because it is a major greenhouse gas. In ruminant nutrition, decreased production of CH_4 can represent an improvement in feed efficiency, because ruminants loose between 2 and 12% of the gross dietary energy (GE) in the form of CH_4 (Johnson and Johnson, 1995). Besides, ruminants contribute to global warming through N excretion from urine and feces. To reduce 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 targeted.

Our hypothesis was that citrus pulp and soybean hulls could replace cereal-based concentrate in goat diets without compromising energy and protein partitioning in lactating goats when mixed diets were formulated with similar amounts of energy and protein. The purpose of the present experiment was to study the effect of substitution of corn grain in the mixed diet by dry citrus pulp or soybean hulls on energy partitioning, CH_4 emissions, C and N balance, and milk performance in dairy goats during mid lactation.

MATERIALS AND METHODS

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 and non-pregnant Murciano-Granadina goats of similar body weight (41.7 ± 2.8 kg BW) and in mid lactation were randomly split into 3 groups (4 goats per treatment) in an incomplete crossover design. Treatments consisted of 3 different concentrate mixed rations (Table 5.1). Goats were fed daily 0.25 kg cereal straw as forage and 1.5 kg of concentrate (forage:concentrate ratio = 14:86). The concentrate was mixed and pelleted along with the premix. One group was fed concentrate with 605 g/kg dry matter (DM) of corn grain (CRG). The other 2 groups substituted corn grain with by-products: dry citrus pulp (CTP) and soybean hulls (SYH). Chemical composition shown in Table 5.1 is whole mixed ration (forage and pelleted concentrate). The mean GE of the 3 diets was 17.6 MJ/kg DM. The main difference among diets was the source of carbohydrates. Starch levels were 41.6, 6.4 and 1.5% (on DM basis) for the CRG, CTP and SYH diets, respectively. NDF and neutral detergent soluble fiber (NDSF) values were 30.1 and 8.5% (respectively, on DM basis) for CRG, 30.1 and 24.1% (respectively, DM basis) for CTP and 59.0 and 17.1% (respectively, DM basis) for SYH. Mixed diets contained similar amounts of CP (15.8%, DM basis). Nutrient requirements followed the recommendation of Lachica and Aguilera (2003) and Calsamiglia *et al.* (2009) for goats in lactation. Apparent total tract digestibility, gas exchange, energy partitioning, C-N balance, oxidation of nutrients, and milk composition and yield were determined. Moreover, ruminal fermentation parameters were determined in the second period of the experiment. Half the daily ration was offered at 08:00 h and half at 16:00 h. Goats had free access to water.

Experimental Schedule and Measurements

The experiment was conducted in an incomplete crossover design in two 31 d periods. During the adaptation, goats were fed with experimental diets in pens for 7 d and then allocated in individual metabolism cages at thermoneutrality (20 to 23 °C determined by a Hobo probe, ONSET data loggers, Cape Cod, MA, USA) for another 7 d. 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 d period, as well as BW at the beginning and end of the period.

Table 5.1. Ingredients and chemical composition of the diets based on corn grain (CRG), dry citrus pulp (CTP) or soybean hulls (SYH).

	Diets[1]		
	CRG	CTP	SYH
Ingredients (g/kg DM)			
Barley straw (as a forage)	150.0	150.0	150.0
Corn	605.2	-	-
Soybean hulls	-	-	610.0
Citrus pulp	-	605.2	-
Soybean meal 44%	202.4	221.7	173.9
Calcium carbonate	21.6	4.0	22.9
Salt	10.8	9.1	25.5
By-pass fat[2]	5.0	5.0	12.7
Premix[3]	5.0	5.0	5.0
Chemical composition (% DM)			
Dry matter (%)	88.36	88.41	90.15
Organic matter	92.80	91.96	89.28
Crude protein	16.66	15.80	14.79
Ether extract	2.66	2.10	2.03
Neutral detergent fiber	30.11	30.08	58.97
Acid detergent fiber	8.55	16.69	37.55
Lignin	0.44	0.93	1.22
NDSF[4]	8.51	24.09	17.07
Non fibrous carbohydrates[5]	45.30	43.98	13.50
Starch	41.63	6.36	1.45
Sugars + pectins[6]	1.75	37.63	12.05
Gross energy (MJ/kg DM)	18.00	17.56	17.21

[1] CRG = corn grain diet; CTP = citrus pulp diet; SYH = soybean hulls diet.
[2] By-pass fat of palm fatty acid distillate. Provided by Norel Animal Nutrition, Norel S.A. (Spain).
[3] Provided by NACOOP S.A. (Spain). Premix composition: Se 40 mg/kg; I 250 mg/kg; Co 80 mg/kg; Cu 3,000 mg/kg; Fe 6,000 mg/kg; Zn 23,400 mg/kg; Mn 29,000 mg/kg; S 60,000 mg/kg; Mg 60,000 mg/kg; vitamin A 2,000,000 IU/kg; vitamin D3 400,000 IU/kg; vitamin E 2,000 mg/kg; nicotinic acid 10,000 mg/kg; choline 20,300 mg/kg.
[4] NDSF = neutral detergent-soluble fiber.
[5] Non-fibrous carbohydrates (NFC) = 100 - (NDF + ash + CP + EE).
[6] Sugars + pectins = NFC - starch.

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 an adequate amount of a solution of H_2SO_4 10% (vol/vol) to acidify the urine of each goat. The acidification of urine was necessary to prevent microbial degradation and the loss of volatile ammonia N. Representative samples (10%) of diet, feces and urine were collected over 5 consecutive days, stored at -20 °C, and pooled for chemical analysis. The goats were milked once daily at 08:00 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 a plastic vial that contained 20 mg of potassium dichromate as a preservative and taken to the Interprofessional Dairy Laboratory of the Valencia Community Region (Valencia, Spain) for compositional analysis. Ruminal fluid samples were collected by stomach tube before the morning feeding on the last day of apparent digestibility trial. Ruminal fluid pH was immediately determined using a Model 265A portable pH meter (Orion Research Inc., Beverly, MA). A ruminal fluid sample was acidified with H_2SO_4 and frozen until later determination of NH_3-N. Samples for analysis of volatile fatty acids (VFA) were mixed with H_3PO_4 and kept frozen until analysis.

Next, gas exchange was measured for each goat during 24 h (4 goats/treatment) by an indirect calorimetric system based on a ventilated head box designed for small ruminants. To this end, 12 d were taken for each period in the incomplete crossover design. The respirometry system has a head hood, a flowmeter (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). CH_4 and CO_2 concentrations were measured using the infrared principle and O_2 by the paramagnetic principle (Easyflow gas analyzer, model 3020, ABB, Alzenau, Germany). Although the unit was an autocalibrated model, the analyzer was calibrated with reference gases before each test. Fernández *et al.* (2012) described the mobile open-circuit respirometry system used for these measurements.

The whole system was calibrated injecting pure N_2 and CO_2 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 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.

Chemical Analysis

Feed, feed refusals and feces samples were first dried in a forced air oven at 55 °C for 48 h then ground to pass a 1 mm screen before analysis. Urine and milk was dried by lyophilization. Chemical analyses of the diet, refusals and feces were conducted

according to methods of AOAC (2000) for DM, ash and ether extract (EE). 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 °C for 6 h to determine organic matter (OM). 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 acid detergent fiber (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 sulfite and alpha-amylase. Lignin was determined according to Robertson and Van Soest (1981). The NDSF content was determined according to Hall *et al.* (1997), after adapting the method to the modifications proposed by Martínez-Vallespín *et al.* (2011). The NFC content of diets was calculated by the difference method based on chemical analysis of individual feeds (NRC, 2001) as NFC = 100 – NDF – ash – CP – EE. 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 (alpha-amylase obtained from Sigma-Aldrich, Steinheim, Germany) according to Batey (1982). The C and N were analysed 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).

The 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.

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 39.54 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):

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

where gases were expressed in L and N_{urine} in g. The body tissue energy (RE_{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 -

(1.7 × CH$_4$), according to Fahey and Berger (1988). The calculations were carried as follow:

$$OXP \ (kJ) = 6.25 \times N_{urine} \times 18.42$$

$$OXCHO \ (kJ) = (-2.968 \times O_2 + 4.174 \times CO_{2x} - 2.446 \times N_{urine}) \times 17.58$$

$$OXF \ (kJ) = (1.719 \times O_2 - 1.719 \times CO_{2x} - 1.963 \times N_{urine}) \times 39.76$$

Then, the HP from oxidation (HPx) was:

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

where gases were expressed in L and N_{urine} in g. 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} x 6.25 x 0.774)] / [O_2 – (N_{urine} x 6.25 x 0.957)]. For C and N balance, we followed the equation and values proposed by McLean and Tobin (1987), and the grams retained in protein ($R_{protein}$) and fat (R_{fat}) were calculated.

The efficiency of use of ME for lactation (k_l) was calculated according to AFRC (1993). If the goat is in positive tissue energy balance, some of the MEI is being directed towards tissue energy retention and therefore MEI is corrected [AFRC, 1993; value for efficiency of utilizing MEI for gain (k_g) is 0.6]. When the goat is in negative energy balance, some of the E_{milk} is derived from body stores and therefore E_{milk} is corrected [efficiency of utilizing body stores for milk production (k_t) is 0.84 according to AFRC, 1993]. Therefore, corrected E_{milk} was regressed on corrected MEI using a linear regression: k_l was the slope of the line and the ME for maintenance (MEm) denotes the value of MEI when E_{milk} is zero. Net energy for lactation (NE$_L$) was computed as MEI x k_l.

Statistical Analyses

The effects of corn substitution with by-products on intake, digestibility, ruminal fermentation parameters, milk yield, energy and C-N balances and oxidation of nutrients were analyzed using the PROC GLM of SAS (2001). The experiment was conducted as an incomplete crossover design and the model for the dependent variables included the fixed effect of diet and period and their interaction. The following statistical model was used: Y = μ + D + T + D x T + ε, where Y is the dependent variable, μ is the overall mean, D and T are the fixed effects of diet and period, respectively; D x T is the fixed interaction effect of diet x period; and ε is the random error. Effects were declared significant at P < 0.05 and P-values between 0.05 and 0.10 were considered as a trend. Tukey tests were used for multiple comparisons among diets and Student's t-test was used for the period.

RESULTS AND DISCUSSION

Feed Intake, Digestibility and Rumen Fermentation

Intake and apparent total tract digestibility of nutrients by Murciano-Granadina dairy goats are shown in Table 5.2. DM, NDF and starch digestibility were significantly ($P < 0.05$) affected by the period, being higher during the second digestibility than the first. No effect of interaction (diet x period) was found, with the exception of starch. In relation to the effect of the diet, DM intake was similar for the 3 diets (1.54 kg/d, on average). Diet affected ($P < 0.001$) the apparent total tract digestibility of most nutrients, as expected from a change in diet, which involves reducing starch and increasing fiber. The higher level of undigestible material for diet SYH compared with CRG and CTP (59% NDF, DM basis for SYH, and 30% NDF, DM basis for CRG and CTP, on average) appeared to be the main factor responsible for the decrease of DM, OM, NFC and GE apparent digestibility in SYH. Consequently, goats fed high concentrate diets are often associated with greater DM and OM and lower NDF digestibility (Kawas *et al.*, 1991).

Rumen fermentation parameters obtained are shown in Table 5.3. 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. The highest level of starch on diet CRG was the cause of the lower pH of CRG than CTP and SYH diet (6.9, 7.4 and 7.3, respectively). Goats fed with more starch also had greater and significant ($P < 0.05$) NH_3-N concentration (24.59 mg/dL for CRG) than other diets (12.69 and 17.74 mg/dL for CTP and SYH, respectively). Ariza *et al.* (2001) also obtained higher NH_3-N concentration via the starch diet than high NDSF diet; 14.2 *vs.* 9.3 mg/dL in hominy feed *vs.* dry citrus pulp diets, respectively, in continuous culture.

Differences were found in most of the VFA studied. Greater ($P < 0.05$) total VFA was found for diet SYH than CRG and CTP (33.15 mM for SYH *vs.* 22.74 mM for the average of CRG and CTP), suggesting more fermentation activity for diet SYH than others and accompanied by greater NDF and ADF digestibility. An increase ($P < 0.05$) in acetic acid in the rumen was observed when fiber amount of diets increased (62.9, 68.7 and 71.2 mol/100 mol for diet CRG, CTP and SYH, respectively). Conversely, the greater starch level of CRG diet increased the production of propionic acid compared with the other diets (15.3 mol/100 mol for CRG *vs.* 13.4 mol/100 mol for the average of CTP and SYH) and also butyric acid (15.1 mol/100 mol for CRG *vs.* 9.8 mol/100 mol for the average of CTP and SYH), although differences were not significant.

Table 5.2. Body weight, dry matter intake and apparent digestibility coefficients of Murciano-Granadina goats (n = 24) during mid lactation according to the type of diet.

	Diets[1]			Periods[2]		SEM[3]	P-value		
	CRG	CTP	SYH	A	B		Diet	Period	Diet x Period
Body weight (kg)	41.8	42.0	41.4	41.8	41.7	0.59	0.949	0.970	0.258
Dry matter intake (kg/d)	1.51	1.53	1.57	1.52	1.55	0.011	0.136	0.198	0.674
Digestibility (%)									
Dry matter	77.4[a]	77.6[b]	71.3[a]	74.4	76.4	0.77	0.001	0.049	0.133
Organic matter	79.9[b]	80.7[b]	74.0[a]	77.2	79.2	0.78	0.001	0.066	0.164
Crude protein	74.6[c]	70.8[b]	64.7[a]	68.9	71.1	1.06	0.001	0.050	0.134
Ether extract	70.7	69.9	75.3	69.8	74.1	1.67	0.449	0.228	0.107
Neutral detergent fiber	59.8[a]	65.5[a]	74.0[b]	64.2	68.7	1.73	0.001	0.048	0.352
Acid detergent fiber	49.8[a]	73.4[b]	75.3[b]	63.3	69.0	3.19	0.001	0.135	0.489
Neutral detergent soluble fiber	57.1[a]	83.1[c]	76.8[b]	72.5	72.2	2.62	0.001	0.857	0.609
Non fibrous carbohydrates	94.6[b]	94.9[b]	84.0[a]	91.3	90.9	1.10	0.001	0.747	0.591
Starch	99.5[c]	98.8[b]	93.3[a]	95.9	98.4	0.70	0.001	0.001	0.001
Gross energy	78.5[b]	79.4[b]	73.6[a]	76.4	77.9	0.73	0.001	0.160	0.072

[a-c] Means within a row with different superscripts differ (P < 0.05).
[1] CRG = corn grain diet; CTP = citrus pulp diet; SYH = soybean hulls diet.
[2] A = first period; B = second period.
[3] SEM = standard error of the mean for diets.

Table 5.3. pH, ammonia N (NH_3-N) and volatile fatty acids of Murciano-Granadina goats (n = 24) during mid lactation according to the type of diet.

	Diets[1]			SEM[2]	P-value
	CRG	CTP	SYH		Diet
pH	6.88	7.44	7.33	0.171	0.061
NH_3-N (mg/dL)	24.59[b]	12.69[a]	17.74[a]	3.447	0.048
Total VFA[3] (mM)	25.31[a]	20.17[a]	33.15[b]	3.773	0.029
VFA (mol/100 mol)					
Acetic acid	62.94[a]	68.69[b]	71.19[b]	2.441	0.035
Propionic acid	15.34	13.12	13.65	0.671	0.181
Butyric acid	15.06	9.80	9.70	1.772	0.075
Isobutyric acid	2.37	3.74	2.12	0.504	0.223
Isovaleric acid	2.88	3.53	2.25	0.370	0.249
η-Valeric acid	1.28	0.99	0.86	0.124	0.437
η-Caproic acid	0.11	0.13	0.22	0.034	0.564

[a-b] Means within a row with different superscripts differ ($P < 0.05$).
[1] CRG = corn grain diet; CTP = citrus pulp diet; SYH = soybean hulls diet.
[2] SEM = standard error of the mean.
[3] VFA = volatile fatty acids.

Energy Balance

The average value obtained for the calibration factor, by releasing a known volume of N_2 or CO_2 into the respirometry system, was 1.0056 ± 0.00158 and 0.9924 ± 0.00915 for O_2 and CO_2, respectively.

Daily energy balance obtained with the 3 diets is shown in Table 5.4. A significant effect of period of time ($P < 0.05$) was observed in HP. The MEI, HP and RE_{body} were significantly affected ($P < 0.05$) for the interaction diet and period. Regarding the effect of diet, no statistically significant differences were observed for GE intake (1,649 kJ/kg $BW^{0.75}$, on average) but energy losses in feces were higher for SYH than CRG and CTP; in fact, GE digestibility of SYH was approximately 5.4% lower than other diets ($P < 0.05$). Therefore, we noted a reduction in digestible energy when feeding goats decreasing amounts of starch and soluble fiber. Urine energy losses were not different among treatments. Both CTP and SYH presented similar energy losses in CH_4 (118 kJ/kg $BW^{0.75}$, on average) and were significantly higher ($P < 0.001$) than CRG (84 kJ/kg $BW^{0.75}$), indicating that increasing the level of starch in diet reduces the CH_4 production, as indicated by different authors and reviewed by Grainger and Beauchemin (2011).

However, Romero-Huelva *et al.* (2012) found a reduction in CH_4 production (from 110 to 67 kJ/kg $BW^{0.75}$) when 35% of cereal-based concentrate was replaced with feed blocks containing wastes of tomato and cucumber. Those authors concluded that the feed blocks might also have the presence of secondary plant compounds, which could act as a safe and natural antimethanogenic additive. Bava *et al.* (2001) did not observe significant differences, but the values of energy losses as CH_4 were 131 and 115 kJ/kg $BW^{0.75}$ for Saanen goats at mid lactation fed a silage-based forage diet *vs.* a non forage diet. López and Fernández (2013) found no significant differences with mid lactation Murciano-Granadina goats, obtaining an average value of 99 kJ CH_4/kg $BW^{0.75}$ when fed 2 mixed diets that differed in carbohydrate source (corn grain or by-products) and starch level.

The MEI was ($P < 0.05$) greater for diet CRG than SYH (1,193 *vs.* 1,079 kJ/kg $BW^{0.75}$, respectively), whereas CTP showed an intermediate value (1,147 kJ/kg $BW^{0.75}$). The HP was higher ($P < 0.05$) for the more fibrous diet than the more starchy diet (908 *vs.* 843 kJ/kg $BW^{0.75}$ for SYH and CRG, respectively). Lachica *et al.* (1997) found that the act of eating accounts for an energy cost of about 0.05 of the MEI for long-dried forage in goats, with similar values between species, so the cost of eating is higher in fibrous diets. The E_{milk} presented no statistically significant differences among diets (400 kJ/kg $BW^{0.75}$, on average).

The energy balance was negative for all treatments, indicating a mobilization of energy from reserves. Nevertheless, the higher starch content of the CRG diet decreased ($P < 0.01$) energy losses of tissues (-23.4 kJ/kg $BW^{0.75}$) compared with CTP and SYH diet (-184.8 kJ/kg $BW^{0.75}$, on average). The main reason this negative energy balance was found in our study is due to the quality of the forage and the level of feeding. The forage:concentrate ratio in our study was 14:86, with a low amount of forage (cereal straw) and of poor quality. Moreover, assuming an MEm of 401 kJ/kg $BW^{0.75}$ (Aguilera *et al.*, 1990), the value of level of feeding in our trial was 3.0, 2.9 and 2.7 for CRG, CTP and SYH, respectively, lower than the recommendation for highly productive ruminants (ranging from 2 to more than 4 times maintenance, according to NRC, 2001). With our high concentrate diets we expected greater DMI and, therefore, larger amounts of MEI, but it was not possible to reach higher DMI with the by-product diets. In ruminants, lipogenic nutrients originate either from fiber that stimulates the ruminal production of acetate and butyrate or from dietary fat, or are derived from body reserves. Glucogenic nutrients originate from starch escaped from rumen degradation or gluconeogenesis and include propionic acid, glucogenic amino acids and lactic acid (van Knegsel *et al.*, 2007). Lipogenic nutrients, which increase milk fat yield, increase the partitioning of ME into milk and consequently decrease the partitioning of ME into body reserves.

Table 5.4. Daily energy partitioning (kJ/kg $BW^{0.75}$) of Murciano-Granadina goats (n = 24) during mid lactation according to the type of diet.

Item[4]	Diets[1]			Periods[2]		SEM[3]	P-value		
	CRG	CTP	SYH	A	B		Diet	Period	Diet x Period
GEI	1,659.9	1,628.6	1,657.4	1,629.3	1,667.9	23.56	0.866	0.486	0.239
E in feces	357.3[a]	336.0[a]	438.7[b]	386.0	368.7	13.28	0.019	0.502	0.656
E in urine	25.7	27.6	22.5	32.7	17.8	3.57	0.890	0.083	0.304
E in CH_4	84.0[a]	118.1[b]	117.5[b]	103.2	109.9	4.51	0.001	0.198	0.096
MEI	1,192.9[b]	1,146.8[ab]	1,078.7[a]	1,107.5	1,171.5	21.80	0.033	0.063	0.018
HP	842.7[a]	862.5[ab]	907.8[b]	845.1	896.9	11.62	0.041	0.016	0.032
E in milk	373.6	445.0	379.9	435.2	363.8	21.45	0.281	0.092	0.391
RE_{body}	-23.4[b]	-160.7[a]	-208.9[a]	-172.9	-89.2	30.71	0.006	0.070	0.045

[a-b] Means within a row with different superscripts differ (P < 0.05).
[1] CRG = corn grain diet; CTP = citrus pulp diet; SYH = soybean hulls diet.
[2] A = first period; B = second period.
[3] SEM = standard error of the mean for diets.
[4] GEI = gross energy intake; MEI = metabolizable energy intake; HP = heat production; RE_{body} = energy retained in body tissues, calculated as RE_{body} = MEI - HP – E in milk.

These observations suggest that glucogenic nutrients (as in the CRG diet) stimulate body fat deposition and the partitioning of ME into body tissue, so that less negative energy balance was observed. As we mentioned previously, despite of the differences among diets in negative energy retention, no significant differences were found in E_{milk}.

The efficiency of use of ME for milk production obtained by regression was 0.59 (corrected E_{milk} = -368.56 + 0.591 x corrected MEI; R^2 = 0.86; residual standard deviation = 60.2). Different authors obtained greater values, such as Aguilera *et al.* (1990) with lactating Granadina goats (k_l = 0.67) or Bava *et al.* (2001), who 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. Those authors found variability when combining different stages of lactation with feed intake level, one of the reasons for differences in efficiencies being related to the complexity of biochemical transformation in tissues that are being synthesized or mobilized. López and Fernández (2013), with mixed diets and goats at mid lactation and positive energy balance, found values around 0.63. Therefore, when we estimate the MEm we found values higher (624 kJ/kg $BW^{0.75}$) than previous authors; Aguilera *et al.* (1990) found values of 401 kJ/kg $BW^{0.75}$ for Granadina goats and Tovar-Luna *et al.* (2010) found MEm values of 431 kJ/kg $BW^{0.75}$, on average. In our study, most of the lactating goats were in negative energy balance and some of the reasons for the dissimilar findings for level of body fat reserves in goats were related to basal metabolism to support milk secretion.

Moreover, when a linear model is used to regress energy retained and MEI, the intercept of the relationship and the MEm are determined using data obtained with animals receiving different MEI and they are then considered to be constant, irrespective of the ME supply to the animal. Therefore, the traditional assumption of a constant MEm that is independent of the MEI may be wrong. In the review of Kebreab et al. (2003), using non linear regression approach with 652 lactating dairy cows, the calculated values of MEm were higher (between 590 and 620 kJ/kg $BW^{0.75}$, with average k_l values of 0.55) than AFRC (1993) and NRC (2001) recommendations (490 and 510 kJ/kg $BW^{0.75}$ for MEm and k_l values of 0.62 and 0.64, respectively).

NE_L was expressed as MJ/kg DM and no significant differences were found when substituting corn with fibrous by-products, although a lower value was found in soybean hulls diet (7.7 in CRG, 7.3 in CTP and 6.6 in SYH). Diets were isonergetic, and most of the variation observed in NE_L was due to changes from starch to fiber (NDF). Bava *et al.* (2001) with goats at mid lactation, found values of 8.5 and 7.7 MJ/kg DM for silage-based and non forage diets, respectively. The average value obtained by López and Fernández (2013) in dairy goats at mid lactation was 7.5 MJ/kg DM when corn grain was replaced by a blend of soybean hulls and corn gluten feed.

Oxidation of Nutrients

The CO_2 production is derived from nutrients oxidation and rumen fermentation. The separation between these 2 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 5.5. The significant ($P < 0.05$) effect of period was related to the stage of lactation. Although both periods were mid lactation, first period was closer to the peak of lactation than the second period. Thus, higher oxidation of fat and lower RQnpx were found during the first period, and greater oxidation of carbohydrates and RQnpx were observed during the second period. Effect of interaction was significant ($P < 0.05$) for fat and carbohydrates oxidation and RQnpx, and this interaction was not studied.

Diet had no significant effect on HPx (853 kJ/kg $BW^{0.75}$, on average). However, more fibrous diets (CTP and SYH) were accompanied by greater ($P < 0.001$) HPf than the starch diet (CRG). A significant difference ($P < 0.05$) was observed for RQnpx, being significantly lower for SYH (0.77) than CTP (0.81), and CRG (0.79) did not differ from the other 2 diets but in all cases was lower than 1. Chwalibog *et al.* (1997) reported that RQnpx lower than 1 indicates predominance of OXF *vs.* OXCHO, as we found in our study.

Due to the negative energy balance of goats, OXF was higher than OXCHO in all diets. The heat from OXP contributed about 8% of HPx on average for 3 treatments. The CTP diet oxidized 58.2% of nutrients as OXF, but only 34.3% as OXCHO. However, the nutrient oxidation of OXF increased significantly ($P < 0.05$) to 74.4% in SYH, and nutrient oxidation of OXCHO decreased significantly ($P < 0.05$) to 18.5%. The higher amount of NDSF in the CTP diet promotes the higher OXCHO compared with the SYH diet. Nevertheless, the CRG diet presented intermediate values compared with the other 2 diets. Few studies relating to oxidation of nutrients are available for ruminants, and especially for small ruminants. López and Fernández (2013) found higher oxidation of nutrients in OXCHO than OXF in goats in mid lactation because the goats in their experiment were in a positive energy balance. Chwalibog *et al.* (1997), in calves with positive retained energy as fat, reported that part of OXF should originate from ingested carbohydrate, mainly fiber.

Table 5.5. Heat production from oxidation and fermentation, daily oxidation of protein, carbohydrate and fat (kJ/kg $BW^{0.75}$) and their contribution to the heat production from substrates oxidation (%) of Murciano-Granadina goats (n = 24) during mid lactation according to the type of diet.

Item[4]	Diets[1]			Periods[2]		SEM[3]	P-value		
	CRG	CTP	SYH	A	B		Diet	Period	Diet x Period
HPx	829.2	843.5	888.9	828.5	879.2	11.49	0.059	0.017	0.030
HPf	13.5[a]	19.0[b]	18.9[b]	16.6	17.7	0.73	0.001	0.198	0.096
OXP	72.4	63.5	62.7	68.4	64.0	2.96	0.443	0.551	0.966
OXCHO	218.3[ab]	288.7[b]	168.3[a]	133.0	317.2	28.88	0.044	0.001	0.055
OXF	538.3[a]	491.1[a]	657.7[b]	626.9	497.8	28.95	0.010	0.003	0.014
OXP/HPx	8.7	7.5	7.1	8.3	7.3	0.35	0.202	0.220	0.906
OXCHO/HPx	26.3[ab]	34.3[b]	18.5[a]	16.1	36.6	3.39	0.024	0.001	0.037
OXF/HPx	65.0[ab]	58.2[a]	74.4[b]	75.6	56.1	3.34	0.018	0.001	0.028
RQnpx	0.79[ab]	0.81[b]	0.77[a]	0.76	0.82	0.010	0.022	0.001	0.030

[a-b] Means within a row with different superscripts differ (P < 0.05).

[1] CRG = corn grain diet; CTP = citrus pulp diet; SYH = soybean hulls diet.

[2] A = first period; B = second period.

[3] SEM = standard error of the mean for diets.

[4] HPx = heat production from oxidation of nutrients; HPf = heat production of fermentation, calculated as HPf = HP - HPx (Brouwer, 1958); OXP = oxidation of protein; OXCHO = oxidation of carbohydrates; OXF = oxidation of fat; RQnpx = non protein respiratory quotient from oxidation of nutrients, calculated as RQnpx = [CO_{2x} – (N_{urine} x 6.25 x 0.774)] / [O_2 – (N_{urine} x 6.25 x 0.957)], where N_{urine} = N in urine.

Carbon and Nitrogen Balance

The daily C and N balance and the protein and fat retained in tissues are displayed in Table 5.6. No effect of interaction between diet and period was observed. Effect of period was significant ($P < 0.05$) in C losses in CO_2, retained N in tissues and $R_{protein}$, following the same tendency as the energy balance. In relation to diet, not significant differences were observed in C intake among treatments (38.6 g/kg $BW^{0.75}$, on average), but C losses in feces were 2.2 g/kg $BW^{0.75}$ greater in SYH than CRG and CTP due to the lower digestibility of DM of this diet. C in urine tended ($P = 0.056$) to be higher with the CTP diet due to higher digestibility of DM and OM together with soluble fiber fractions (NDSF and NFC). Following the trend observed for CH_4 energy, the C losses in CH_4 were significantly lower with CRG due to lower CH_4 production with this diet. C losses in CO_2 show the same differences among diets as C in CH_4. C secreted into the milk was not significantly affected by treatment. The ratios between milk C output and C ingested were 0.21, 0.24 and 0.20 for CRG, CTP and SYH, respectively. The CTP diet showed greater efficiency, with more C retained in milk in terms of intake. The 3 groups had negative balances, although CRG diet had less negative balance (-0.42 g/kg $BW^{0.75}$; $P < 0.05$) than SYH diet (-3.94 g/kg $BW^{0.75}$, on average).

Goats of 3 groups ingested similar amounts of N (2.36 g/kg $BW^{0.75}$, on average). The N losses in feces were greater for SYH treatment (0.81 g/kg $BW^{0.75}$) when compared with CRG and CTP (0.66 g/kg $BW^{0.75}$, on average). N losses in urine and N into milk were not affected by treatment. Therefore, the ratios between milk N output and N ingested were similar among groups and presented an average value of 0.27. The N balance was different ($P < 0.05$) between CRG and SYH, although CTP was not different from the others. The N balance was positive for all treatments. Although some authors (Kebreab *et al.*, 2010) indicated a reduction in urinary N output when MEI increased; in our study, greater and non significant values of urinary N were found for the CRG diet (diet with higher MEI). This was probably due to greater NH_3-N found in CRG and, consequently, more urea N excreted in urine. Therefore, this numerically higher urinary N excretion in diet based on corn would indicate an inefficient use of ruminal NH_3-N for ruminal proteosynthesis, even when rapidly degraded starch was available for ruminal microorganisms.

The values of N retained in the body were converted to grams of protein. Differences ($P < 0.05$) were found between CRG and SYH, whereas CTP showed a middle value. Therefore, $R_{protein}$ was 3.5, 2.7 and 1.9 g/kg $BW^{0.75}$ for CRG, CTP and SYH, respectively. Regarding R_{fat}, the CTP and SYH diets showed greater ($P < 0.05$) fat mobilization (-6.0 g/kg $BW^{0.75}$, on average) than CRG (-2.9 g/kg $BW^{0.75}$).

Table 5.6. Daily C-N balance (g/kg BW$^{0.75}$) of Murciano-Granadina goats (n = 24) during mid lactation according to the type of diet.

Item[4]	Diets[1]			Periods[2]		SEM[3]	P- value		
	CRG	CTP	SYH	A	B		Diet	Period	Diet x Period
C balance									
C intake	37.87	39.36	38.62	38.19	39.04	0.548	0.583	0.502	0.237
C in feces	8.41^a	8.50^{ab}	10.65^b	9.00	9.36	0.319	0.027	0.584	0.898
C in urine	1.02	1.39	1.12	1.16	1.19	0.058	0.056	0.802	0.844
C in CO_2	19.47^a	20.92^b	21.06^b	19.37	21.59	0.348	0.019	0.001	0.093
C in CH_4	1.52^a	2.13^b	2.12^b	1.86	1.99	0.082	0.001	0.198	0.096
C in milk	7.87	9.29	7.62	8.75	7.77	0.453	0.278	0.280	0.423
C retained body	-0.42^b	-2.89^{ab}	-3.94^a	-1.97	-2.87	0.608	0.023	0.376	0.079
N balance									
N intake	2.46	2.35	2.28	2.33	2.39	0.039	0.184	0.480	0.235
N in feces	0.63^a	0.69^a	0.81^b	0.73	0.69	0.021	0.001	0.219	0.735
N in urine	0.63	0.55	0.54	0.59	0.56	0.026	0.443	0.551	0.966
Metabolizable N	1.20	1.10	0.93	1.01	1.15	0.050	0.061	0.133	0.318
N in milk	0.64	0.66	0.63	0.68	0.61	0.027	0.868	0.195	0.774
N retained body	0.56^b	0.44^{ab}	0.30^a	0.33	0.54	0.046	0.034	0.010	0.430
$R_{protein}$	3.50^b	2.74^{ab}	1.89^a	2.03	3.39	0.287	0.034	0.010	0.430
R_{fat}	-2.93^b	-5.62^a	-6.42^a	-3.95	-6.04	0.702	0.039	0.081	0.057

$^{a-b}$ Means within a row with different superscripts differ (P < 0.05).
[1] CRG = corn grain diet; CTP = citrus pulp diet; SYH = soybean hulls diet.
[2] A = first period; B = second period.
[3] SEM = standard error of mean for diets.
[4] Metabolizable N = N_{intake} - N_{feces} - N_{urine}; $R_{protein}$ = retained protein; R_{fat} = retained fat.

Milk Production

Table 5.7 reports the main productive results of the goats in the experiment. No effect of period of time was found in milk yield and composition. However, an effect of interaction was observed for DM, fat and protein of milk. Diet had no effect on milk yield; average milk yield was 1.72 kg/d. However, a numerically higher milk yield was found for CTP than CRG and for CRG than SYH. Increased milk fat content is common when dietary fiber concentrations increase at the expense of starch. Significant differences ($P < 0.01$) were observed for milk fat between SYH and CRG (6.57 and 4.95%, respectively) and, consequently milk DM was also higher (16.06 and 14.02% for SYH and CRG, respectively). This was because the SYH diet had greater fiber content (Table 5.1), greater NDF and ADF digestibility (Table 5.2), and higher values of acetic acid in rumen fluid than CRG (Table 5.3). The depression in milk fat with CRG diet can be 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). Moreover, milk fat content was higher for goats fed fibrous diet and with greater fat mobilization (Table 5.6). Milk protein content differed between SYH and CTP (4.16 and 3.44%, respectively). Lactose content was not statistically different among treatments (4.55%, on average).

Methane Emissions

The effect of diet and period on CH_4 emissions are shown in Table 5.8. No effect of period and its interaction with diet was found. Goats fed CTP and SYH diets produced similar CH_4 emissions (34.8 g/d, on average) and were significantly higher ($P < 0.05$) compared with goats fed CRG diet (24.7 g/d). The type of carbohydrates in the diet influenced the proportion of individual VFA formed in the rumen and thus, the amount of CH_4 produced. According to Johnson and Johnson (1995), 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 between CTP and SYH can be explained by the fact that CTP, although with a lower NDF content, has considerable amount of pectins, which, similarly to hemicellulose and cellulose, are fermented to VFA and primarily to acetic acid, which promotes methanogenesis.

Methane emission as a percent of MEI and in g/kg DMI was greater ($P < 0.001$) for CTP and SYH diets (10.6% and 22.4 g/kg, on average) than the CRG diet (7.0% and 16.4 g/kg). Methane conversion ratio, also called Ym factor, represents energy loss as CH_4 per unit of GE intake. CH_4 conversion ratio for CTP and SYH was 7.2%, on average, and CRG showed a value of 5.1% ($P < 0.001$). Kreuzer *et al.* (1986) also found significantly lower CH_4 loss (total as well as percent of GE) on rations with native starch than rations with cellulose.

Table 5.7. Daily milk production and composition of Murciano-Granadina goats (n = 24) during mid lactation according to the type of diet.

	Diets[1]			Periods[2]		SEM[3]	P-value		
	CRG	CTP	SYH	A	B		Diet	Period	Diet x Period
Milk production (kg/d)	1.75	1.92	1.48	1.86	1.58	0.088	0.129	0.091	0.356
Milk composition (%)									
Dry matter	14.02[a]	14.78[ab]	16.06[b]	15.21	14.70	0.283	0.006	0.260	0.015
Fat	4.95[a]	5.90[ab]	6.57[b]	6.15	5.46	0.226	0.006	0.071	0.046
Protein	3.74[ab]	3.44[a]	4.16[b]	3.63	3.93	0.113	0.009	0.064	0.013
Lactose	4.53	4.61	4.51	4.57	4.54	0.044	0.760	0.774	0.857

[a-b] Means within a row with different superscripts differ (P < 0.05).
[1] CRG = corn grain diet; CTP = citrus pulp diet; SYH = soybean hulls diet.
[2] A = first period; B = second period.
[3] SEM = standard error of mean for diets.

Table 5.8. Methane emission of Murciano-Granadina goats (n = 24) during mid lactation according to the type of diet.

Item[4]	Diets[1]			Periods[2]		SEM[3]	P- value		
	CRG	CTP	SYH	A	B		Diet	Period	Diet x Period
g CH_4/d	24.68[a]	35.09[b]	34.42[b]	30.41	32.38	1.334	0.001	0.231	0.392
Ym (%)	5.06[a]	7.28[b]	7.09[b]	6.34	6.61	0.282	0.001	0.412	0.497
CH_4/MEI (%)	7.02[a]	10.33[b]	10.90[b]	9.36	9.48	0.462	0.001	0.785	0.516
g CH_4/kg DMI	16.37[a]	22.96[b]	21.92[b]	19.97	20.86	0.836	0.001	0.408	0.489
g CH_4/kg OM digested	22.00[a]	30.93[b]	33.20[b]	28.42	29.01	1.298	0.001	0.663	0.593
MJ MEI/g metabolizable N	1.03	1.05	1.17	1.12	1.04	0.035	0.227	0.254	0.835
g CH_4/kg milk	15.46[a]	18.47[ab]	23.46[b]	17.26	21.00	1.174	0.008	0.052	0.257

[a-b] Means within a row with different superscripts differ (P < 0.05).
[1] CRG = corn grain diet; CTP = citrus pulp diet; SYH = soybean hulls diet.
[2] A = first period; B = second period.
[3] SEM = standard error of mean for diets.
[4] Ym = methane energy/gross energy intake; MEI = metabolizable energy intake; DMI = dry matter intake; OM = organic matter.

Although CH_4 emission is most commonly expressed in the literature relative to GE intake, the most meaningful expression is relative to intake of digestible OM because of its high correlation with ruminally fermented OM. On these bases, ruminal CH_4 emission was also greater (P < 0.001) with CTP and SYH as compared with CRG. The CH_4 production per kg digested OM was 10.1 g higher in by-product diets, on average, than CRG.

When we report the CH_4 production related to the energy to protein ratio we found the same tendency. Thus, this ratio is MEI divided by metabolizable N [MJ/g = MEI / (N_{intake} − N_{feces} − N_{urine})] and the values obtained were 1.03, 1.05 and 1.17 for CRG, CTP and SYH respectively. This means more CH_4 was produced when more MEI was available per unit of metabolizable N. Bava *et al.* (2001) found the same trend with lactating Saanen goats comparing a non forage diet with a forage diet; as more CH_4 was emitted, more energy to N ratio was observed (1.42 *vs.* 1.33 MJ/g N for the forage and non forage diet, respectively). As Dijkstra *et al.* (2013) indicated, integration between protein and energy metabolism is essential, and energy and protein should be considered together rather than as 2 distinct entities. When the relationship between CH_4 production and milk production was compared we found that goats fed SYH produced 8.0 g CH_4/kg milk more (P < 0.01) than goats fed CRG; conversely, the CTP diet presented intermediate values.

CONCLUSIONS

Our study 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 61% of corn in the diet by dry citrus pulp or soybean hulls did not affect milk yield (1.72 kg/d) and more fat content was observed for fibrous diets (6.57, 5.90 and 4.95% in SYH, CTP and CRG, respectively). The MEI was greater for diet CRG than SYH (1,193 *vs.* 1,079 kJ/kg $BW^{0.75}$, respectively), CTP showed an intermediate value (1,147 kJ/kg $BW^{0.75}$). The HP was higher for more-fibrous diet than more-starchy diet (908 *vs.* 843 kJ/kg $BW^{0.75}$ for SYH and CRG, respectively). The efficiency of use of ME for milk production obtained by regression was 0.59. Replacement with fibrous by-products increased methane emissions. Greater CH_4 was produced with dry citrus pulp and soybean hulls diets (34.8 g/d on average) than the corn diet (24.7 g/d). The higher starch diet resulted in more energy to be partitioned into the body, whereas the fibrous diet split more energy into milk fat. Hence, lactating goats could use dry citrus pulp and soybean hulls diets without detrimental effect on milk performance, although attention should be paid to the quality of forage and level of feeding.

ACKNOWLEDGEMENTS

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GENERAL DISCUSSION

The feeding is the most important cost in a farm since it may represent between 60 and 80% of total costs, hence the importance of adjusting the feeding to the animal requirements. To make this adjustment is necessary to know both the energetic needs of animal as the energy content of diet and its utilization by the animal, which vary depending on various factors such as the physiological state of the animal, the final destination of the energy, etc.

This Thesis was planned with the objective of developing an entire methodology with which to perform these studies. We proposed to design, build and validate an indirect calorimetry system to perform nutritionals trials. The calorimetric technique was chosen since, if one is able to design precise equipment, this technique is one of the best methods to determine the heat production of the animals, and so estimate the energy efficiency. Furthermore, if gas analyzer has a cell to measure methane, as our system, it would be possible to quantify the amount on methane exhaled by the animal during ruminal fermentation, and evaluate the environmental consequences.

The aim was to design an economical and portable system, so the mobile open-circuit respirometry system was built initially connected to a mask and short-term measures in time were conducted. Further this system was improved by replacing the mask by a head hood that would allow making measurements sustained for longer periods of time and not prevent the animal from eating. The mobile indirect calorimeter system was adjusted to ranges of gas exchange of small ruminants, calibration factors were determined in order to evaluate leaks from the system and, the whole open circuit calorimeter system was compared against C and N balance.

Then some nutritional applications were conducted in sheep and goats at different physiological stages, and total heat production (HP) was determined. As the use of mixed diets is common in Spain, the substitutions of cereal grains of the diet by several by-products were evaluated. Cereal grains, principally of corn and barley, are usually present in ruminant diets and that could cause competition with the human food in the future. Thus, in this Thesis corn and barley grains were replaced by other products obtained from the industry as soybean hulls, corn gluten feed or dry citrus pulp. The latter by-product is highly important in the area where this Thesis was carried out. It was studied the effect of the replacement of cereal grain by those by-products on energy and C-N balances and substrate oxidation; and milk performance in lactating animals.

The results of each of the experiments in this study have been presented in their respective chapters, each of which included specific discussions of the topics investigated. This General Discussion integrates the main aims of the study and discusses them from a broader perspective.

PROPER FUNCTIONING OF DESIGNED CALORIMETRIC SYSTEM

The technical requirements for an indirect calorimeter system are strict. The need to measure small changes in gas concentration enforces a high demand on the accuracy and precision of the instrumentation necessary. As outlined by Ferrannini (1988), these include:

1) an airtight system with appropriate flow to give O_2 and CO_2 concentrations within the linear range of the analyzers
2) sensitive and stable O_2 and CO_2 analyzers for continuous measurements of expired air
3) a method to remove moisture from the expired air before analysis
4) computer hardware and software to capture and analyze the data
5) a calibration method using standard gas mixtures

As presented in our study, all these conditions are supplied in the indirect calorimeter system designed and set up during experimental phase of this thesis.

By far the most difficult aspect of indirect calorimetry is the measurement of O_2. Moreover, the abundance of O_2 (20.95%) relative to CO_2 (0.03%) in ambient conditions dictates that measurement errors of O_2 have greater implications on the calculations. The most limiting factor involved in the accurate determination of O_2 consumption is the precision with which gas volumes and compositions can be measured (Miller and Koes, 1988). The precision of the equipment is a guarantee of reliable results. The CO_2 and CH_4 analyzers of our equipment have a range of 0 to 1.5% and 0 to 0.15%, respectively, and operate on infrared principle; whereas O_2 analyzer works on a paramagnetic principle with a range of 19 to 21% and it was equipped with an atmospheric compensation module to account changes in pressure.

In last years, several papers have used indirect calorimetry systems to study energy metabolism of small ruminants. These have utilized masks (El-Meccawi *et al.*, 2008), hoods (Patra *et al.*, 2009; Puchala *et al.*, 2012) or whole-animal chambers (Rapetti *et al.*, 2005; Nielsen *et al.*, 2014). The mobility of these units may be limited, especially the whole-animal chambers which are totally immobile. Moreover, it has been suggested that HP data from measurements using the mask technique may require careful interpretation due to it can cause discomfort and affect the gas measurements. The system described and improved in this Thesis is advantageous because of its accuracy, simplicity and mobility. Also, this indirect calorimetry system is flexible: it may be used with a variety of species including sheep, goats, deer, pigs, poultry, rabbits and cattle. The limiting factor in the use of this system for different species is the hood design, regulation of appropriate air-flow rates and range of gases in the analyzer.

A way to validate the constructed calorimetry system would be to compare the data obtained by it with data obtained by other method. As discussed in the General Introduction, HP can be determined by indirect calorimetry (RQ method) but other methods exist to determine it, as CN method. In general, in the literature not significant

differences are found between RQ and CN methods for different species. So, Fernández *et al.* (2012) showed similar HP values, obtained by both methods, in Manchega sheep fed different nutrition levels. Christensen *et al.* (1988), in 3 experiments with pigs of different body weights and fed different diets or feeding level, presented similar HP values between methods although in some cases significant differences were obtained. In small animals, Zhao *et al.* (1997) not found differences statistically significant between the two methods for HP in rats independently of temperature and diet composition.

All those authors generally not showed significant differences between HP values calculated according to the RQ method compared to the CN method, but slightly higher values are systematically found when using the RQ method. Therefore, the obtaining of similar values between both methods may be a proof of the proper operation of designed system. We have collected all data of HP obtained in this Thesis with our indirect calorimetry system connected to head hood (experiments from II to V), and presented in this General Discussion. In Figure 6.1, HP values determined by RQ method (HP-RQ) were compared with the HP values calculated by CN method (HP-CN).
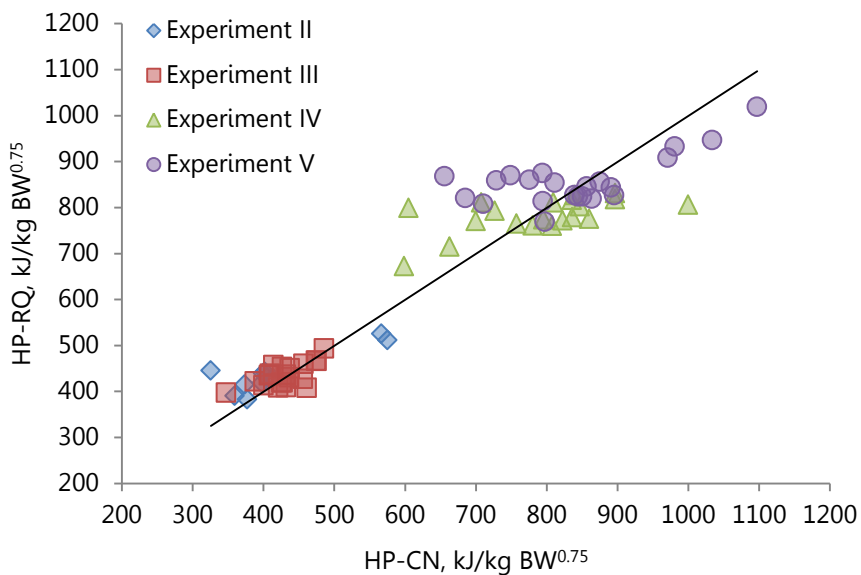
Figure 6.1. HP calculated by indirect calorimetry (RQ method; HP-RQ), using the calorimeter connected to head hood described in this Thesis, in relation of HP estimated from C and N balances (CN method; HP-CN).

A linear regression was conducted with data of HP represented in Figure 6.1 and the equation of this regression shown a relationship between both methods very close to 1 (HP-RQ = 0.99 ± 0.011 x HP-CN; n = 80; average = 658.3; RMSE = 67.09; R^2 = 0.90),

which indicate that HP obtained with RQ and CN methods is almost identical. On the other hand, represented values in this Figure are grouped in 2 zones, which coincide with HP of dry sheep (experiments II and III) and HP of lactating goats (experiments IV and V). In the case of dry sheep, average HP was 436.7 and 425.9 kJ/kg $BW^{0.75}$ for RQ and CN methods (P = 0.886), respectively; and lactating goats produced 821.9 and 816.2 kJ HP/kg $BW^{0.75}$ by RQ and CN methods (P = 0.987), respectively. These results agree with those of the literature; HP values obtained in experiments of this Thesis were slightly higher with RQ method compared with CN method. With these results we can assure the correct operating of our calorimetry system.

ESTIMATION OF ENERGY REQUIREMENTS OF SHEEP AND GOATS

One of the principal aims of the calorimetric studies applied to small ruminants is estimate the energy requirements of these, with the purpose of providing feeding recommendations once known the energy value of food. Consequently, we will try to estimate the energetic needs of sheep and goats from data presented in experiments of this Thesis.

The response of energy recovery of a productive animal to ME intake is often represented as a 2 stage linear model. The breakpoint between both phases, when retained energy (RE) is null, is assumed to be an estimate of ME for maintenance (MEm) whereas slopes for ME intake bellow or above MEm represent efficiencies of using ME (k) for maintenance and production, respectively. In this conventional model, the intercept of the relationship, of which the absolute value is equivalent to the extrapolated HP at zero energy intake (net energy for maintenance; NEm), and MEm are determined using data obtained with animal receiving different ME intake and they are then considered to be constant, irrespective of the ME supply to the animal. Nevertheless, fasting heat production (FHP) after a fed period, which is indicative of NEm, depends of the previous feeding level. Therefore, the traditional assumption of a constant MEm that is independent of ME intake may be wrong. The distinction between catabolic and anabolic stages (for ME lower or higher than MEm) is arbitrary and does not reflect biological mechanism; the transition between both stages may be more progressive than a breakpoint between two linear phases.

In order to estimate energy requirements of sheep, in Figure 6.2 is represented the relationship between RE and MEI of the sheep. Data obtained for Manchega and Guirra breed sheep (experiments II and III, respectively) have been processed together, since feeding level was similar for both breeds. RE was regressed linearly against MEI and the line is presented in the same Figure.
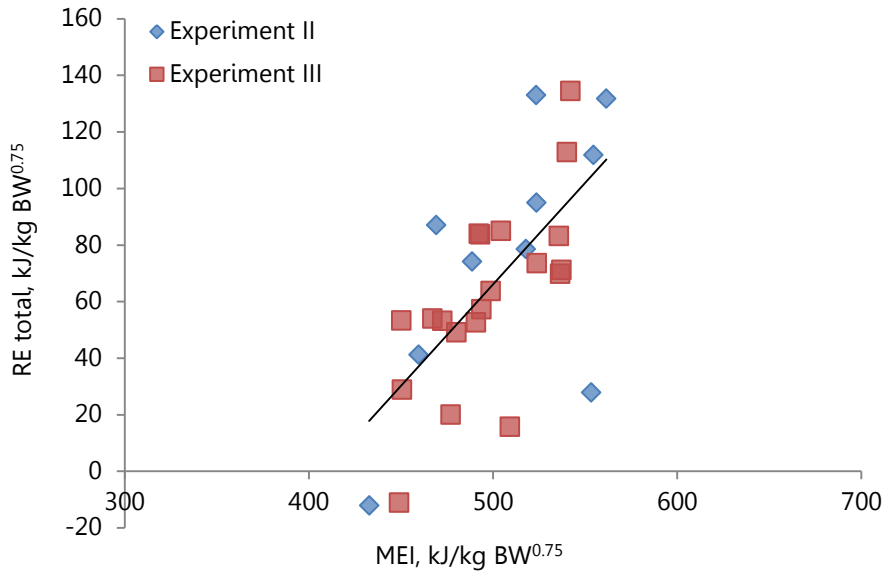
Figure 6.2. Representation of the relationship between RE and MEI, expressed in kJ/kg BW$^{0.75}$ of Manchega and Guirra sheep (experiments II and III of this Thesis, respectively). The line shows the linear regression between RE and MEI.
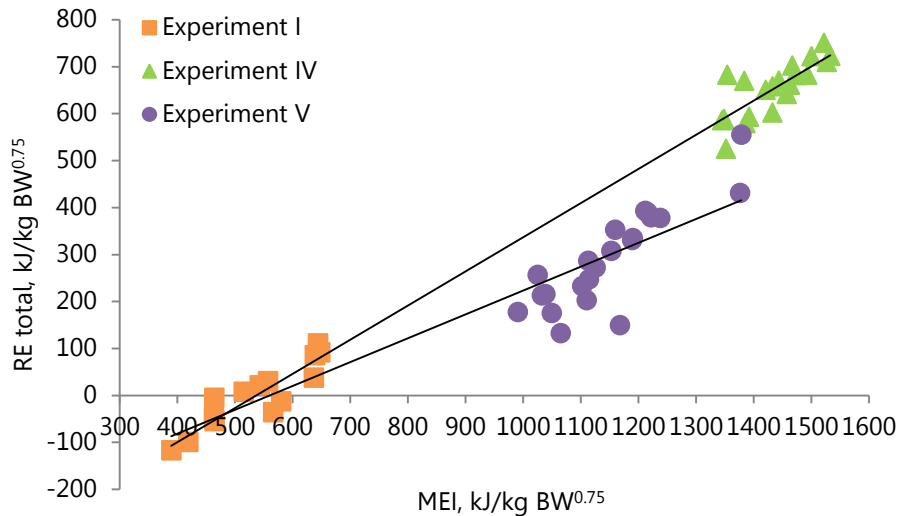

Figure 6.3. Representation of the relationship between RE and MEI, expressed in kJ/kg BW$^{0.75}$ of Murciano-Granadina goats (experiments I, IV and V of this Thesis). The lines show the linear regression between RE and MEI of experiment I with other two.

Milk energy yield plus apparent body energy retention or loss was regressed to MEI and is showed in Figure 6.3. In this approach we use linear regression. The two experiments with lactating Murciano-Granadina goats (experiments IV and V) were analyzed separately because of the differences in energy intake among goats of both experiments. In this Figure also is represented RE and MEI of goats of experiment I, whose feeding level was close to maintenance. Two analysis of linear regression were carried out of RE against MEI for goats of experiments I and IV firstly, and experiments I and V secondly, whose lines are drawn in the Figure.

By linear regressions of RE against MEI we have estimated energy requirements of sheep and goats, which are resumed in Table 6.1. The intercept of equation describe NEm and the slope show the efficiency of ME utilization. MEm was obtained by equating to zero the regression equation.

Table 6.1. Estimation of energy requirements for maintenance (kJ/kg $BW^{0.75}$) and global efficiency (k) for sheep and goats studied in the experiments of this Thesis.

Experiment	n	Regression equation	R^2	NEm	k	MEm
II and III (sheep)	36	RE = 0.719±0.149 MEI - 293±75	0.455	293	0.72	408
I and IV (goats)	34	RE = 0.727±0.013 MEI - 390±15	0.990	390	0.73	537
I and V (goats)	38	RE = 0.508±0.030 MEI - 284±29	0.893	284	0.51	560

NEm = net energy for maintenance; k = efficiency of utilization of metabolizable energy; MEm = metabolizable energy for maintenance.

Many factors can influence energy metabolism of animals, it vary among and within animals and depend on many factors including species, age, sex, physiological state of production, diet or intake level, environmental conditions, or activity. The values of MEm obtained for goats are higher than those for sheep, which is justified on the basis that while sheep values were determined on non-productive animals at lower feeding level, those for goats were obtained on lactating animals. The difference of MEm between the goats of experiments IV and V is due to different feeding levels. It is recognized that one of the most widely acknowledged effect of MEm is level of feed intake, so, at levels of intake below MEm, FHP is minimized because of decreased basal metabolic rates, for which splanchnic tissues can account for an appreciable portion (Freetly *et al.*, 1995; Wester *et al.*, 1995; Sainz and Bentley, 1997). Moreover, high levels of intake can affect MEm, depending on the method of partitioning. That is, HP or energy expenditure by "support" tissues, such as splanchnic bed, increases with increasing metabolic workload associated with rising feed intake (Johnson and Johnson, 1995).

In Table 6.2. are exposed energy requirements for maintenance and global efficiencies for sheep and goats estimated by several Feeding systems. The most important and widely used Feeding systems are shown. Those systems are: the Institut National de la Recherche Agronomique (INRA, 2007), the Agricultural and Food Research Council (AFRC, 1998), the National Research Council (NRC, 2007) and, only for sheep and cattle, the Commonwealth Scientific and Industrial Research Organisation (CSIRO, 2007). As mentioned above, an elevated number of factors influence energy requirements. Feeding systems show variable energetic needs into species, since the methods to estimate those needs or other variables as breed or productive status of animals vary among systems. So, the NEm requirements in sheep are lowest for AFRC and CSIRO than INRA and NRC (see Table 6.2). The first two systems are based on the results of the fasting metabolism studies of ARC (1980). The other two systems acknowledge that HP under fasting conditions involves decreased metabolic rates for energy conversion. For this reason, NRC system adopted an extrapolated value of fasting metabolism and INRA system based its requirements on values obtained by various techniques (slaughtering measurements, feeding trials, calorimetry studies, blood indicators).

Table 6.2. Maintenance energy requirements (kJ/kg BW^{0,75}) of sheep and goats and their efficiencies by different Feeding systems.

Feeding systems	NEm	k	MEm
Sheep			
INRA (2007)	235	0.56 - 0.64	369 - 417
AFRC (1998)	217	0.64 - 0.75	326 - 379
NRC (2007)	234	0.73	322
CSIRO (2007)	217 - 252	0.65 - 0.76	287 - 390
Goats			
INRA (2007)	265	0.56 - 0.64	428 - 488
AFRC (1998)	347	0.64 - 0.75	462 - 542
NRC (2007)	347	0.64 - 0.75	423 - 542

NEm = net energy for maintenance; k = efficiency of utilization of metabolizable energy; MEm = metabolizable energy for maintenance.

Our data indicate high demands of MEm for sheep and goats respect to recommendations given by appointed Feeding systems. Average MEm estimated for Manchega and Guirra breed sheep is 408 kJ/kg $BW^{0.75}$, which is in the range of energy needs given for INRA (that contemplate dairy sheep into their Feeding system), but overcome data for sheep of other systems (based on meat or hair sheep). However, higher values than obtained in our Thesis, were found in the literature. So, Nsahlai *et al.* (1997) estimated a MEm for Ethiopian Menz sheep of 422 kJ/kg $BW^{0.75}$ and, Nielsen *et al.* (2014) for Shropshire sheep estimated an average value of 438 kJ/kg $BW^{0.75}$. In goats we found an estimated MEm value of 537 (experiment IV) and 560 kJ/kg $BW^{0.75}$ (experiment V). Experiment IV are in the range of AFRC and NRC but experiment 5 is out of range, probably because the energy balance during the trial was negative and the RE used in the regression include the energy mobilized from tissues to milk energy, I mean, it was not corrected to estimate MEm.

By other hand, we observe the same tendency (greater values in goats than sheep) than in the literature. One of the reasons is that on traditionally worldwide Feeding systems (AFRC, CSIRO, INRA, NRC) the sheep values were generally obtained on animal at maintenance feeding level, while those for goats were obtained on lactating animals.

The efficiency of use of MEm obtained in sheep (0.72) is similar to this given by the majority of Feeding systems and data reported in the literature, though higher than efficiencies displayed by INRA. When NEm is estimated, we find that net energy requirements of our sheep (293 kJ/kg $BW^{0.75}$) are slightly higher than these shown by most important ruminants Feeding systems (Table 6.2). As can be seen in Figure 6.2, some animals had consumed 561 kJ/kg $BW^{0.75}$, namely 153 kJ/kg $BW^{0.75}$ more than maintenance estimation of 408 kJ/kg $BW^{0.75}$. In our studies sheep were fed above maintenance, hence positive energy balance, therefore is not possible estimate accurately MEm. So, we have used intakes higher than 1/3 above maintenance to estimate energy requirements for maintenance, and due that this values were not corrected to zero energy balance, we overestimate the MEm.

The estimated MEm for goats of experiment IV (537 kJ/kg $BW^{0.75}$) is in the range of requirements given by AFRC and NRC systems (from 423 to 542 kJ/kg $BW^{0.75}$), but goats of experiment V had greater MEm requirements (560 kJ/kg $BW^{0.75}$) than those of experiment IV and values ranges given by Feeding systems (Table 6.2). Luo *et al.* (2004) estimated a MEm for Angora goats very close to that obtained in experiment IV, this value was of 533 kJ/kg $BW^{0.75}$. And Tovar-Luna *et al.* (2010), for Alpine goats in lactation fed 60% concentrate, presented a MEm of 522 kJ/kg $BW^{0.75}$.

The different energy balance between goats of experiments 4 and 5 affected efficiency of energy utilization. Goats of experiment V had a negative energy balance, therefore, part of used energy proceed from body reserves energy mobilization and other part of food (the distribution of energy from body tissues or food has not been studying in this Thesis). Goats of experiment IV, with positive energy retention, showed a k of 0.73 that was in the range given by AFRC and NRC systems (0.64-0.75; Table 6.2),

but k of goats of experiment V (0.51) was lower that efficiencies found in the literature, since part of the energy proceed of body tissues and it would be used with lower efficiency that energy from food.

Finally, goats of experiment IV had an estimated NEm of 390 kJ/kg $BW^{0.75}$, higher that shown by Feeding systems. And goats of experiment 5 had an estimated NEm (284 kJ/kg $BW^{0.75}$) lower than experiment IV, due to negative energy balance during lactation. This value is between NEm given by INRA and AFRC or NRC.

Therefore, the efficiencies of conversion of ME to NE vary depending on the type of requirements (maintenance, lactation, growth) and on the system considered. AFRC and INRA estimate these efficiencies with equations that use metabolizability (ME/GE) of the diet as independent variable. CSIRO (no goats) uses the concentration of ME in the diet (called M/D). AFRC and CSIRO use separate equations for maintenance and milk production. INRA use only one NE unit and adopted an equation for both maintenance and milk production, and this choice were justified by the similar slopes of k_m and k_l. The NRC does not clearly state which efficiency was used to convert NE for maintenance and for lactation to ME.

Moreover, we have to keep in mind that our regression approaches were done with the whole data set from each experiment and, data were not corrected to zero energy balance, the milk energy content was not corrected for energy mobilization from tissues and the regression do not use determined FHP as intercept.

Therefore, this higher estimation seems that linear regression is not appropriate tool for estimating MEm when animals from different feeding level were used to estimate maintenance. Over last few decades, the different Feeding systems had analyzed energy balance data with rigid acceptance of linear methods and maintenance requirements were corrected to zero energy balance and lactation requirements were corrected to zero body fat mobilization. Our result shown that alternatives to linear regression should be useful to analyze energy balance data from small ruminants, although that is not the scope of the present Thesis and this topic should be investigated in the future. Having more data should be interesting to evaluate key parameters in the current ME or NE system for lactating dairy sheep and goats. For instance, classical studies based on 600 dairy cow observations (Moe *et al.*, 1972) reported maintenance requirements of 490 kJ ME/kg $BW^{0.75}$ and other mathematical approaches as Kebreab *et al.* (2001) based on dataset of similar size, ranged values of MEm between 340 and 640 kJ/kg $BW^{0.75}$. This show the wide possibilities of a mobile indirect calorimetry system as a tool to collect energy balances data from small ruminants and thus be able to elaborate a dataset for future investigation on energy metabolism.

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CONCLUSIONS

The conclusions of this Thesis are:

1. The open-circuit indirect calorimetry system designed and built in this Thesis is advantageous with respect to other calorimetry systems because of its mobility and versatility. It could be used more under field conditions than respiration chambers.

2. The evaluated calorimetric system has proven to be very accurate to measure respirometry gas exchange of small ruminants, since the average value obtained for the calibration factor of O_2 was 1.005 ± 0.0101, which confirms the absence of leaks from the whole system.

3. The differences in HP obtained between indirect calorimetry and carbon and nitrogen balance are 2 and 1% for sheep and goats, respectively, denoting that our system determine heat production of reliable and precise way.

4. Methane emission of dry and non-pregnant sheep was 18.2 g/d and it of lactating goats was 30.7 g/d.

5. Net energy requirements for maintenance of Guirra and Manchega breed sheep, determined on this Thesis by lineal regression or measurement of fasting heat production, respectively, were 270 kJ/kg $BW^{0.75}$.

6. The efficiency of use of metabolizable energy for lactation in Murciano-Granadina goats fed mixed diets, calculated on this Thesis, was 0.61.

IMPLICATIONS

By this system can be conducted a lot of calorimetry trials with small ruminants, sheep and goats. On the one hand, much is the information that can be obtained on the energy requirements of small ruminants. There is not an only value of energy needs by each animal species because of these depend of many factors. It is necessary to study how different factors such as breed, physiological state, level of production, activity, locomotion or thermal stress, affect these requirements.

Also, calorimetry trials can be carrying out with small ruminants fed locally available feed, and to accumulate data for development of farm systems and farmers in these different areas. Moreover, the study of utilization of many products or by-products obtained of these areas in small ruminants feeding will concluded advantages and disadvantages of their use in energy balances.

On other hand, ruminants are one important source of emission of methane. Methane is a potent greenhouse gas that is released into the atmosphere mainly by respiration of ruminants. So, via the calorimetry system described in this Thesis, which permits to measure concentrations of methane in the air, can be tested different strategies to reduce enteric methane emissions. Besides, methane not only represents an environmental issue, it is also an energy loss for the animal. These two aspects are the main driving forces for conducting research about enteric CH_4 emission.

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CURRICULUM VITAE

Curriculum Vitae

María del Carmen López Luján was born on 1st of July 1983 in Valencia, Spain. In December 2004, she graduated from Agricultural Technical Engineering at Universitat Politècnica de València (UPV) and subsequently, in June 2009, she reached the degree of Agricultural Engineering at the same university. In December of the same year she concluded her MSc. in Animal Production at Animal Science Department of UPV. Throughout her university studies she collaborated with Group of Animal Production Systems and Technology of Institute for Animal Science and Technology of UPV. This collaboration was focused on ovine production and led to a series of scientific publications and contributions to conferences.

From July 2009 she joined contractually at the Animal Nutrition Group of the same Institute of UPV as a technician, and her field of work expanded to ovine and caprine species. In 2012 she started her PhD financed by Conselleria d'Educació, Cultura i Esport from Generalitat Valenciana. The result of this grant is the current dissertation.

List of Publications

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