Thèse / *Thesis*

requise pour
l'obtention du Titre

submitted
for the Degree of

Master of Science

EFFECTS OF L-CARNITINE SUPPLEMENTATION ON DAIRY GOATS UNDER HEAT STRESS CONDITIONS

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Zaragoza, Octubre 2015

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de la Nutrition Animale
Spanish Foundation for the Development of Animal Nutrition

ACKNOWLEDGEMENTS

"Aw-iddand-wi it-yefen, ad-ye ṭṭ$_3$anad atyaweḍ; wama win id-nemsawa,ur tes$_3$iḍwiiy-irefdenwayeḍ".

I hope to have friends wiser than me, and try to learn from them; because having friends such as myself, nobody would learn from other.

(AitMenguellat, 1993)

I would like to start by thanking Neus Torrent for providing me with help, unconditional support, always listened to my concerns and celebrated my triumphs. Secondly I want to thank Samantha Love who gave so much help with the farm work. Next I would like to thank my supervisor Ahmed Salama for guiding me through the project and allowing me to develop my scientific skills throughout; Gerardo Caja for the push up he gave me throughout the wrighting part.

Lastly, I would like to thank my family and friends specially Wellington Coloma, who supported me through this challenging year.

Abstract

The objective of the study was to evaluate the effects of rumen-protected L-carnitine on productive and metabolic variables in dairy goats under thermo-neutral (TN) and heat stress (HS) conditions. Two brands of L-carnitine were used: Lohman (LOH; CarnEon 20 Rumin-Pro, Lohmann Animal Health, Germany) and Sintal (SIN; Carnitina 20%, Sintal, Vicenza, Italy). In a first experiment, the ruminal degradability of both products was evaluated in situ using nylon bags in a cannulated dairy cow. The degradability was evaluated twice when the cow was fed 100% lucerne hay ad libitum or 20% lucerne hay and 80% concentrate. The effective degradability of DM was higher for high forage compared to high concentrate diet (85.8 vs. 72.8%). The opposite results were observed for CP according to diet (85.6 vs. 95.4%). When feeding the same diet, both L-carnitine products had similar degradability for DM and CP. In a second experiment, 6 Murciano-Granadina dairy goats (2.48 ± 0.02 L/d of milk yield; 46.1 ± 0.5 kg BW and 134 ± 2 DIM) were used in a 6 × 6 Latin square design with 6 periods of 16 d each. Goats were allocated to one of 6 treatments in a 3 × 2 factorial arrangement. Factors were 1) diet: control (CON) and supplementation with of L-carnitine (1 g/d) from LOH or SIN; and 2) ambient conditions: TN (15 to 20°C; THI = 59 to 65) or HS (0900 to 2100 h at 35°C, 2100 to 0900 hat 28°C; 45 ± 5% relative humidity; THI = 82 and 77). This resulted in 6 treatment combinations: TN-CON, TN-LOH, TN-SIN, HS-CON, HS-LOH and HS-SIN. L-carnitine supplementation had no effect on the measured variables in TN or HS conditions. The HS goats tended ($P < 0.06$) to eat larger particle sizes compared to TN goats. Compared to TN, HS goats lost more ($P < 0.05$) subcutaneous fat as indicated by the difference in fat thickness measured at the start and end of each period (−0.72 vs. +0.64 mm). This would indicate body fat mobilization despite the fact that blood non-esterified fatty acids and β-hydroxybutyrate did not vary between TN and HS goats. Despite the high rumen degradability of L-carnitine products, blood carnitine fractions (free-, acetyl- and total-carnitine) increased ($P < 0.001$) by supplementation. In conclusion, supplemented L-carnitine to dairy goats was absorbed, but no lactational effects were observed under thermo-neutral or heat stress conditions. Fat metabolism under heat stress conditions deserves more research as the results of fat mobilization indicators in blood and changes in subcutaneous fat thickness were contradictory.

Keywords: L-carnitine, metabolism, heat stress, rumen degradability, goats.

Resumen

El objetivo del estudio fue evaluar los efectos productivos y metabólicos de la suplementación con L-carnitina protegida en cabras lecheras bajo condiciones de termo-neutralidad y estrés por calor. Dos productos comerciales de L-carnitina fueron usados: Lohman (LOH; CarnEon 20 Rumin-Pro, Lohmann Animal Health, Alemania) y Sintal (SIN; Carnitina 20%, Sintal, Vicenza, Italia). En el 1er experimento, la degradabilidad ruminal de los dos productos fue evaluada in situ usando bolsas de nylon en una vaca lechera canulada. La degradabilidad fue evaluada dos veces, cuando la vaca fue alimentada con 100% heno de alfalfa ad libitum o 20% alfalfa y 80% concentrado. La degradabilidad efectiva de la materia seca fue mayor en la ración alta en forraje comparada con la ración alta en concentrado (85.8 vs. 72.8%). Resultados opuestos fueron encontrados para la proteína bruta (85.6 vs. 95.4%). Para la misma ración (80% concentrado), los dos productos de L-carnitina tuvieron la misma degradabilidad de MS y de PB. En el 2o experimento, 6 cabras lecheras murciano-granadinas (2.48 ± 0.02 L/d de leche; 46.1± 0.5 kg PV and 134 ± 2 DEL) fueron usadas en un diseño cuadrado latino 6 x 6 con 6 periodos de 16 d cada uno. Las cabras fueron colocadas en 1 de los 6 tratamientos en un arreglo factorial 3 x 2. Los factores fueron 1) ración: control (CON) y suplementación con L-carnitina (1 g/d) de LOH o de SIN; y 2) condiciones ambientales: TN (de 15 a 20ºC; THI = de 59 a 65) o HS (de 0900 a 2100 a 35ºC, de 2100 a 0900 h a 28ºC; 45 ± 5% humedad relativa; THI = 82 y 77). Esto resultó en 6 combinaciones de tratamientos: TN-CON, TN-LOH, TN-SIN, HS-CON, HS-LOH y HS-SIN. La suplementación con L-carnitina no tuvo efecto en los parámetros medidos en condiciones de TN y HS. Las cabras en HS tendieron ($P < 0.06$) a consumir partículas de tamaño más grande comparado con las cabras bajo condiciones de TN. Comparado con TN, las cabras HS perdieron más ($P < 0.05$) grasa subcutánea, lo cual fue detectado por la diferencia del espesor de la grasa medido al inicio y al final de cada periodo (-0.72 vs. +0.64 mm). Esto podría indicar movilización de grasa corporal a pesar de que los ácidos grasos no esterificados y el β-hydroxybutirato en sangre no variaron entre las cabras bajo condiciones de TN y HS. A pesar de la alta degradabilidad ruminal de la L-carnitina, sus fracciones en sangre (libre-, acetil- y total-carnitina) incrementaron ($P < 0.001$) con la suplementación. En conclusión, la L-carnitina suplementada a las cabras fue absorbida, pero no hubo efectos sobre la producción de leche bajo condiciones de termo-neutralidad y estrés por calor. Es necesaria más investigación en metabolismo de grasa bajo condiciones de estrés por calor, ya que los indicadores sanguíneos de movilización de grasa y los cambios en el grosor de grasa subcutánea fueron contradictorios.

Palabras claves: L-carnitina, metabolismo, estrés por calor, degradabilidad ruminal, cabras.

Résumé

L'objectif de l'étude était d'évaluer les effets L-carnitine protégée du rumen sur les variables de production et métaboliques chez les chèvres laitières dans des conditions de stress thermique (HS) et de thermo-neutralité (TN). Deux marques de L-carnitine ont été utilisés: Lohmann (LOH; CarnEon 20 Rumin-Pro, Lohmann Animal Health, Allemagne) et Sintal (SIN; Carnitina 20%, Sintal, Vicenza, Italie). Dans une première expérience, la dégradabilité ruminale des deux produits a été évalué in situ en utilisant des sacs en nylon dans une vache laitière canulée. La dégradabilité a été évaluée deux fois lorsque la vache a été alimentée à 100% foin de luzerne ad libitum ou 20% foin de luzerne et 80% de concentré. La dégradabilité effective de DM était plus élevé pour la ration fourragère par rapport à la ration de concentré élevé (85,8 vs 72,8%). Des résultats opposés ont été observées pour le CP selon la ration (85,6 vs 95,4%). Lorsque, une même ration était offerte, les deux produits L-carnitine avaient une dégradabilité similaire pour la DM et la CP. Dans une seconde expérience, 6 Murciano-Granadina chèvres laitières (2,48 ± 0,02 L/j de production laitière; 46,1 ± 0,5 kg BW et 134 ± 2 DIM) ont été utilisés dans un 6 × 6 carré latin avec 6 périodes de 16 d chaque unes. Les chèvres ont été attribuées à l'un des 6 traitements dans un arrangement factoriel 3 × 2. Facteurs étaient 1) alimentation: control (CON) et la supplémentation avec de la L-carnitine (1 g/j) de LOH ou SIN; et 2) des conditions environnementales: TN (15 à 20 ° C; THI = 59 à 65) ou HS (0900 à 2 100 h à 35 ° C, 2100-0900 à 28 ° C; 45 ± 5% d'humidité relative; THI = 82 et 77). Cela a abouti à 6 combinaisons de traitement: TN-CON, TN-LOH, TN-SIN, HS-CON, HS-LOH et HS-SIN. L-carnitine n'a eu aucun effet sur les variables mesurées dans des conditions TN ou SH. Les chèvres en HS ont eu tendance ($p < 0,06$) à manger des tailles de particules plus grandes par rapport aux chèvres TN. Par rapport à TN, les chèvres en HS ont perdu plus ($P < 0,05$) graisse sous-cutanée, comme indiqué par la différence d'épaisseur de graisse mesurée au début et à la fin de chaque période (-0,72 vs +0,64 mm). Ceci indiquerait une mobilisation des graisses corporelles en dépit du fait que les acides gras non estérifiés du sang et β-hydroxybutyrate ne varient pas entre les chèvres TN et HS. Malgré la haute dégradabilité ruminale des L-carnitines, fractions de carnitine sanguine (Libres-, acétyl et carnitine et totale) ont augmenté ($P < 0,001$) par la supplémentation. En conclusion, la L-carnitine supplémenté à des chèvres laitières a été absorbé, mais aucun effet sur lactation ont été observés dans des conditions thermo-neutres ni du stress thermique. Le métabolisme lipidiques dans des conditions de stress thermique mérite plus de recherche vue que les résultats des indicateurs sanguins de mobilisation des graisses et les variations de l'épaisseur de graisse sous-cutanée étaient contradictoires.

Mots clefs : L-carnitine, métabolisme, stress thermique, dégradabilitée ruminale, chèvres.

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List of abbreviations

ATP	Adenosine Triphosphate
ACS-ACOD	Method of colorimetric enzymatic test
ADF	Acid detergent fiber
BHBA	β-hydroxybutrate
BW	Body weight
CF	Crud fiber
CoA	Coenzyme A
CP	Crud protein
CPT	Carnitine palmitoyl transferase
DIM	Days in milk
DM	Dry mater
DNA	Deoxyribonucleic acid
ED	Effective degradability
HS	Heat stress
LDL	Low Density Lipoprotein
NAD	Nicotinamide adenine dinucleotide
NDF	Neutral detergent fiber
NEFA	Non-Esterified Fatty Acids
PDH	Pyruvate dehydrogenase
PDIA	Dietary protein undegraded in the rumen which is truly digestible in the small intestine
PDIE	True protein digested in the small intestine when fermentable energy is limiting
PFK-1	Phosphofructo-1-kinase
RH	Relative humidity
SC	Somatic cell
Tdb	Dry bulb temperature
THI	Temperature humidity index
TML	Trimethyllysine
TN	Thermo-neutral
UAB	Autonomous university of Barcelona
UEm	Fill units sheep
UFL	Feed unit system for milk
VFA	Volatile fatty acid

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INTRODUCTION

I. INTRODUCTION

Globally the goat plays an important role in the economy of thousands of livestock owners in different regions as they can be raised under a wide range of terrain and climatic conditions. The climatic change will negatively affect the dairy industry, and the importance of goats to the dairy industry would increase in proportion to the severances of changes in environmental temperatures (Silanikove and Koluman (Darcan), 2015). Under heat stress there are extra maintenance expenditures due to muscle movements for panting, N losses with sweating, increased chemical reactions in the body and the production of heat shock proteins that consume large amounts of ATP (Salama et al., 2014). In parallel with the increase in maintenance requirements, there is a clear reduction in feed intake, which is negatively reflected on animal productivity. Recently, Salama et al. (2015) indicted how heat stress impacts the 5 welfare freedoms (freedom from thirst, hunger and malnutrition, freedom from discomfort, freedom from pain, injury and disease, freedom to express normal behavior, and freedom from fear and distress). Therefore, mitigation strategies are needed to improve welfare and productivity in hot weather conditions. Cooling strategies based on the use of water (sprinklers or fans and sprinklers) are effective in reducing body temperature and enhancing feed intake (Collier et al., 2006). However, steady increases in electricity costs, reduction of water availability, and an increase in water usage regulations might make the use of cooling systems unviable, especially for dairy goats. In this scenario, feeding strategies including the use of feed supplements could be profitable.

Carnitine is an essential substrate for carnitine palmitoyl transferase-I, which is a mitochondrial enzyme that condenses carnitine with fatty acyl-CoA to form acylcarnitine. The carnitine-acylcarnitine system is required for mitochondrial oxidation of fatty acids and energy production. Feed-restricted dairy cows infused with L-carnitine produced greater 3.5% fat-corrected milk yield compared with control animals (Carlson et al., 2006). Additionally, L-carnitine infusion effectively decreased liver lipid accumulation during feed restriction as a result of greater capacity for hepatic fatty acid oxidation. As indicated above, maintenance requirements are increased by heat stress and in the same time feed intake is depressed. Consequently, heat-stressed animals face a challenge to meet their energy needs. Therefore, it seems logical that supplementation with L-carnitine would

improve the fatty acids utilization for energy production and would be beneficial under heat stress conditions.

LITERATURE REVIEW

II. LITERATURE REVIEW

II.1. Heat stress in dairy animals: Effects on metabolism and milk production

There are numerous comprehensive reviews describing the effects of heat stress on ruminant species as well as strategies to mitigate their adverse effects (Fuquay, 1981; Collier et al., 1982, 2006, 2008; Silanikove, 2000; Kadzere et al., 2002; West, 1999, 2003; Hansen, 2004; Marai et al., 2007; Bernabucci et al., 2010; Marai and Haeeb, 2010; Baumgard and Rhoads, 2013; Sevi and Caroprese, 2012; Rhoads et al., 2013, Mader, 2014; Salama et al., 2014). However, in this review the focus will be briefly on the impact of heat stress on metabolism and some performance characteristics in dairy animals, especially goats.

Heat stress (HS) can be simply defined as a condition that occurs when an animal cannot dissipate an adequate quantity of heat, whether it is produced or absorbed by the body, to maintain body thermal balance (Bernabucci et al., 2014).The process of adaptation can be grouped into six categories: (1) anatomical, (2) morphological, (3) physiological, (4) feeding behavior, (5) metabolism, and (6) performance (Silanikove and Koluman, 2015).The skin temperature of the goats follows the ambient temperature change, showing that mechanism of redirecting blood to the skin is one of the physiological adaptation mechanism (Darcan and Güney, 2008). Low metabolic requirements, ability to reduce metabolism, efficiency of utilization of high forage diet (i.e. high digestive efficiency), ability to economize nitrogen requirements, and efficient use of water are also important characteristics which help goats to cope with harsh environmental conditions (Silanikove, 2000).

Endocrine control of nutrient partitioning is likely to be complex with overlapping system involving many synergisms. In goats, little data is available on the effect of nutritional and environmental factors on mammary and adipose tissue metabolism. Glucose and insulin are homeostatic regulators of lipid metabolism in adipose tissue (Bauman and Currie, 1980).Glucose is needed for lactose production in dairy animals, and in high producing animals it can account for 60-85% of total body glucose metabolism (Church,

1988;Bauman and Currie, 1980). Muscles also require glucose to produce glycogen, but ruminant muscles contain less glycogen than other species (Church, 1988).

Propionate is the only VFA which makes a net contribution to glucose synthesis and it is the most important precursor. In sheep fed near maintenance, over 80% of propionate absorbed into the portal blood is utilized for glucose synthesis (Steinhour and Bauman, 1988). Feed intake in HS dairy animals is dramatically decreased, which results in a decrease in the amount of propionate absorbed from the rumen.

Also, amino acids could be used for gluconeogenesis and the production of glucose. The maximum contribution of amino acids to the glucose requirements is about 36%, with 32% coming from the liver and 4 % from the kidney (Bergman, 1983). All amino acids can be used for gluconeogenesis except lysine, leucine and taurine (Church, 1988). Alanine and glutamine are the most glucogenic and account for 40-60% of the glucose produced from amino acids in sheep, and they are released from the skeletal muscle (Cecava et al., 1988). In starved or fasted ruminants, glycerol contributes in much larger portion of glucose synthesis (Church, 1988).

Skeletal muscle is the largest protein pool in the body and by its volume contributes substantively to whole body protein turnover and energetics. Ubiquitin-mediated proteolysis within proteasomes is the most important protein degradation pathway in tissues in general, and particularly responsible for the degradation of myofibrillar proteins in skeletal muscle (Keller et al., 2014). Ubiquitin is a high temperature thermostable globular protein which play important role in DNA repair, embryogenesis, immune defense, transcription, apoptosis and protein degradation. As commented above, under heat stress animals eat less to reduce heat production, and consequently they are in negative energy balance. Nevertheless, they do not mobilize body fat reserves as indicated by the no change in blood non-esterified fatty acid (NEFA) and β-hydroxybutrate (BHBA) levels compared to animals in thermo-neutral conditions in case of dairy cows (Baumgard and Rhoads, 2013) and goats (Hamzaoui et al, 2013; Salama et al., 2014). Instead, heat-stressed animals mobilize body proteins (muscle degradation) and it seems that they use amino acids for gluconeogenesis to meet their requirements of glucose.

Secretion of thyroid hormones is decreased under HS, thereby hampering the productivity of the animal for which optimum levels of thyroid hormones are required (Todini et al., 2007). Furthermore, Sivakumar et al. (2010) reported that there was an increase in plasma concentration of prolactin from 11.7 to 26.4µg/L and cortisol from 25.3 to 40.6 nmol/L in HS goats, whereas T3 and T4 levels decreased from 4.6 and 21.3 to 3.2 and 16.7 pmol/L, respectively. All these changes in hormonal milieu help in reducing heat production, but have a negative impact on milk production.

When lactating Saanen goats exposed to moderate or severe HS for 4 d, their milk yield losses were3% and13%, respectively (Sano et al., 1985). Similarly, relatively low level (6%) of reduction in milk yield was found in Alpine goats in Brazil (Brasil et al., 2000). Hamzaoui et al., (2013) observed no change in milk yield of HS goats at late lactation, with milk of HS goats containing lower protein (−12.5%) and casein (−11.5%) than TN goats. Cows in a shade vs. no shade environment yielded 10% more milk (Collier et al., 2006).We should take into account the breaking point from which HS starts to have an impact on animal production. Bernabucci et al., (2014) observed that in lactating cows, the breaking point for fat yield was 72.Theeffect of high ambient temperatures on production traits shows that the first-parity cows had lower sensitivity to HS than multiparous cows (Bernabucci et al., 2014). Additionally, the negative effect of HS on milk production varies according to stage of lactation, and is more likely to take place in earlier than later stages of lactation in cows (Bernabucci et al., 2010) and goats (Salama et al., 2014; 2015).

Compared with TN goats, HS goats experienced greater rectal temperature (+0.58°C), respiratory rate (+48 breaths/min), water intake (+77%) and water evaporation (+207%) (Hamzaoui et al., 2013).Cows in a shade vs. no shade environment had lower rectal temperatures (38.9 vs.39.4°C), reduced respiratory rate (54 vs. 82 breaths/ min) as shown by (Collier et al. (2006).

II.2. Measurement of Heat Stress Degree: Temperature-Humidity Index:

The temperature humidity index (THI) is expressed as a single value representing the combined effects of air temperature and humidity, which is commonly used to evaluate the

degree of HS in dairy cattle. Dikmen and Hansen (2009) reported 8 equations for the calculation of THI. One of the common equations is that used by the NRC (1971) as follows:

$$THI = (1.8 \times T_{db} + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T_{db} - 26.8)],$$

Where T_{db} is the dry bulb temperature (ºC) and RH is the relative humidity (%). Silanikove and Koluman (2015) proposed THI thresholds for differeent degrees of HS in dairy cows and goats (Table1).

Table 1: Comparison of weather heat stress risk classes between dairy cows and dairy goats (Silanikove and Koluman, 2015).

Heat stress class	Dairy cows	Dairy goats
Normal: no effect on milk yield	THI < 74	THI < 80
Alert: modest effect on milk yield	74 ≤ THI < 79	80 ≤ THI < 85
Danger: sever effect on milk yield	79 ≤ THI < 84	85 ≤ THI < 90
Extreme: can result in death	THI ≥ 84	THI ≥ 90

The THI index indicated previously does not take into account solar radiation and wind speed, which can affect heat load on the animal. Mader et al. (2006) proposed adjustments to the THI for solar radiation (W/m^2) and wind speed (m/s). In case of feedlot beef cattle, Mader et al. (2006) reported that THI values should be reduced by 2.0 units for each 1-$m \cdot s^{-1}$ increase in wind speed and increased by 0.68 units for each 100-$W \cdot m^{-2}$ increase in solar radiation.

II.3. Alleviating Heat Stress Strategies:

Generally, there are 3 main strategies for reducing the negative effects of HS: (1) changing feeding schedule and the use of feed supplements, (2) the use of shading and different cooling systems (fans, sprinklers, fans and sprinklers), and (3) genetic selection of more heat-tolerant animals. Management procedures typically used to avoid negative effects on performance are to increase dietary energy density via increased the concentrate proportion in the die tor supplementation with fats (Collier et al., 2006). Sprayed and

ventilated goats for 1 h a day (from 12.00 to 13.00 h) produced 21% more milk yield and had 18% more food consumption (Darcan and Güney, 2008). It is well known that cooling systems have a very positive effect on alleviating the negative effects of high THI in dairy cattle (Collier et al., 2006). However, water will be limited and more restrictive regulations of its use will be issued in the future because of the climatic change phenomenon. Consequently, the feeding strategies, especially the use of some supplements that could modify animal metabolism, could be a more viable solution. In the current work, L-carnitine was tested due to its role in the energy production at the cellular level.

II.4. Body fat distribution in goats:

The distribution of body fat in goats differs appreciably from that in ewes (Colomer-Rocher et al., 1987). For instance, subcutaneous (SC) fat deposits in the dorsal region are not highly noticeable, and when the body condition score method has used the dorsal and lumbar regions to predict fat stores in goats, the results have not proven to be satisfactory (Hervieu et al., 1991). For this reason, the body condition score has been determined using the sternal region, where large amounts of fat accumulate in goats (Hervieu et al., 1991). Accordingly, in Celtiberica meat goats the best relationships between in vivo and carcass measurements of fat thickness were obtained when measurements were taken at the sternum, and the best anatomical point was located between the third and fourth sternebrae in goats (Mendizabal et al., 2010; Teixeira et al., 2008).

In terms of fat distribution among the different adipose tissues dairy breeds generally lay down more fat in their internal depots (mainly peri-renal and omental), whereas meat breeds deposit higher amounts of subcutaneous fat (Kempster and Southgate, 1984). Subcutaneous fat proved to be the depot with the greatest capacity for the storage and mobilization of body fat (Mendizabal et al., 2007), by both the amount of fat and the adipocyte size (Arana et al., 2005). The distribution of fat depot in goats differs from that in sheep, mainly because inter-muscular fat depots are favored over subcutaneous depots in goats (Morand-Fehr, 1981; Colomeretal., 1987; Teixeira et al., 1995).

II.5. Effect of L-carnitine on dairy animals:

II.5.1. Definition:

Carnitine (l-3-hydroxy-4-N, N, N-trimethylaminobutyrate)has a molecular weight of 161.2g/mol and is a very hygroscopic compound, easily soluble in water. Its name is derived from the fact that it was first isolated from meat (*carnus*). Some described it as an essential metabolite(Bach, 1982), others a non-essential organic nutrient (Harpaz, 2005),which has a number of indispensable roles in intermediary metabolism.

II.5.2. Synthesis, Functions and Kinetics of L–carnitine:

In human, cell culture models suggest that carnitine is transported from the intestinal lumen into the enterocyte, but passes across the serosal membrane into the circulation by simple diffusion. Intracellular acetylation of carnitine may facilitate its diffusion across the serosal membrane (Rebouche, 2004).In tissues and body fluids; L-carnitine is present in free and esterified forms (Alhomida et al., 1995).

L-carnitine has 2 main roles. First, the shuttle activity of carnitine is required as a cofactor for carnitine palmitoyltransferase-I in the transport of long chain fatty acids; the presence of oxidryl group (-OH), who can be reversibly esterified by various acyl radicals(Matera et al., 2003; Carlson et al., 2007) permit the transport of the activated long-chain fatty acids from the cytosol to the mitochondrial matrix, where β-oxidation takes place (Vaz and Wanders 2002; Palomer et al 2013). Secondly, carnitine functions as a buffer for acetyl groups that may be present in excess in different tissues during ketosis and hypoxic muscular activity (Matera et al., 2003) Figure 1.

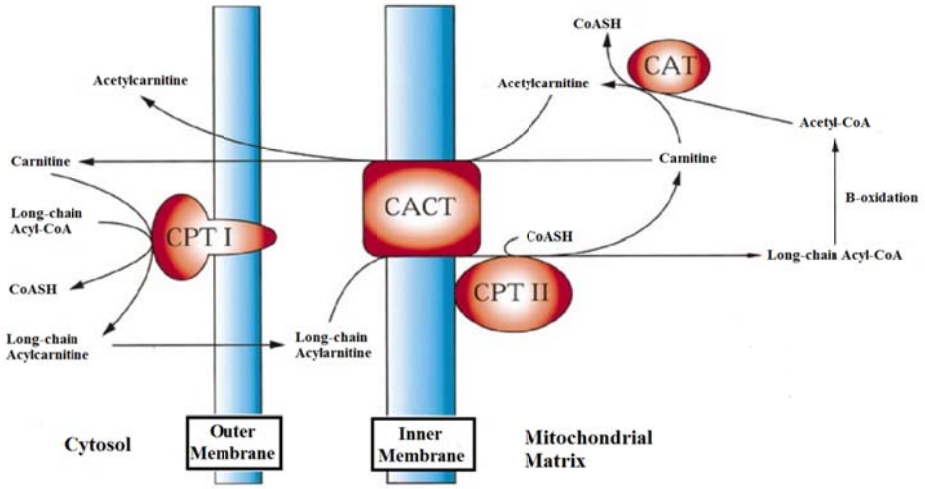


Figure 1: Function of carnitine in the transport of mitochondrial long-chain fatty acid oxidation and regulation of the intramitochondrial acyl-CoA/CoA ratio (Vaz and Wanders, 2002).

L-Carnitine is synthesized from the essential amino acids lysine and methionine with the assistance of vitamin C and other secondary compounds produced in the body (Harpaz, 2005). A unique feature of the pathway is the requirement for methylation only of protein- bound lysine. This process and the accessibility of ε-N-trimethyllysine (TML) to the mitochondrial site of ε-N-trimethyllysine hydroxylase apparently limit the overall rate of carnitine synthesis (Rebouche, 2004). Enzymes catalyzing the several steps in carnitine biosynthesis are ubiquitous, with the exception of γ-butyrobetaine hydroxylase. This enzyme is not found in skeletal muscle and heart, and is most active in liver, kidney, and testis. It is well known, in fact, that in mammals' proteins such as calmodulin, histones, cytochrome c, and myosin contain TML residues. Therefore, it is conceivable that free TML may be released in the course of the protein turnover and, then, utilized for carnitine production, although a direct evidence of this formation route has never been reported (Vaz and Wanders, 2002). In addition, it is of interest that alfalfa leaves, one of the most important forage grasses, resulted one of the richest source of free TML (Servillo et al., 2014).

Betaine and choline methyl groups may increase the synthesis of carnitine and influence body fat deposition (Daily et al., 1998). Methyl groups of choline become

available when choline is oxidized to betaine (Banskalieva et al., 2005). Hepatic synthesis of L-carnitine is stimulated by stress, most of this extra carnitine is acetylated and passes into the blood, urine and milk. This loss of carnitine may represent a major drain on the methyl pool and hence on methionine (Emery et al., 1992).

A study with camels showed the concentration of total carnitine was 71.3 ± 8.0 nmol/ml in plasma (Alhomida et al., 1995). Arabian camel skeletal muscle possessed the highest levels of carnitine. In the heart, carnitine value was significantly lower compared to the muscle (Alhomida et al., 1995). In early lactation dairy cows muscle carnitine remained elevated 7 d after the end of supplementation (Carlson et al., 2007). The hepatic concentration of total carnitine was more or less similar to that of the heart (Alhomida et al., 1995). Mean whole-body turnover time was 66 h. Mean turnover times for compartments with slow turnover (skeletal muscle) and other compartments with relatively rapid turnover (liver and kidney) were 191 h and 12 h, respectively (Rebouche, 2004).On the other hand, kinetics of carnitine transport in vitro and observations in rats in vivo, when extended to humans, suggest that 10–20% of the dose may enter the liver (net uptake) during the first 2 h after dose administration (Bremer, 1983).

In healthy animals the excretion in urine of unchanged carnitine seems to be the main pathway of loss (Bremer, 1983). In human, under normal conditions, only a very small fraction (usually <5%) of filtered carnitine is excreted (Rebouche, 2004). This excretion is increased in thyrotoxic patients and decreased in hypothyroid (Maebashi et al., 1976). A decrease in thyroxin secretion must be considered an important adaptation which helps the body tissues to survive the starvation condition, because low thyroxin concentrations result in low turnover rates and low turnover rates means also low rates of proteolysis and lipolysis. In human, carnitine that is not absorbed in the small intestine is almost completely degraded by indigenous flora of the large intestine (Rebouche, 2004). Excretion of carnitine in milk and urine increased when carnitine was supplemented (LaCount et al., 1995).

Oral supplementation or administration of L-carnitine resulted in increased carnitine levels in plasma, milk, liver and muscles in a dose-dependent manner in cows (LaCount et al., 1996; Cao et al., 2007; Carlson et al., 2006; Carlson et al., 2007

II.6. Role of L-carnitine in the Randle cycle:

Specifically, the Randle cycle (Figure 2) draws attention to competition between glucose and fatty acids for their oxidation in muscle and adipose tissue. It is a biochemical mechanism that controls fuel selection and adapts substrate supply and demand in normal tissues in coordination with hormones controlling substrate concentrations in the circulation. By inhibiting glucose oxidation, fatty acids and ketone bodies are oxidized and contribute to a glucose-sparing effect. In addition, inhibition of glucose oxidation at the level of pyruvate dehydrogenase (PDH) preserves pyruvate and lactate, both of which are gluconeogenic precursors (Garland et al., 1962). Mediation is made by a short-term inhibition of several glycolytic steps, namely glucose transport and phosphorylation, 6-phosphofructo-1-kinase (PFK-1), and PDH (Hue and Taegtmeyer, 2009). L-carnitine is a conditionally essential nutrient that serves as a substrate for a family of acyltransferase enzymes (carnitine palmitoyl transferase CPT1 and CPT2) that catalyze the reversible exchange of acyl groups between CoAand carnitine (Muoio et al., 2012). CPT-1 catalyzes the transfer of long-chain fatty acids from acyl-CoA to carnitine to form acyl-carnitine, which is the rate-controlling step in the mitochondrial fatty acid oxidation pathway (Palomer et al., 2013). When carnitine is lacking, a common feature seems to be the accumulation of triacylglycerol in the cell; under these conditions an inhibited fatty acid oxidation diverts fatty acids from oxidation to esterification (Bremer, 1983). By affecting CPT, glucose oxidation may be enhanced.

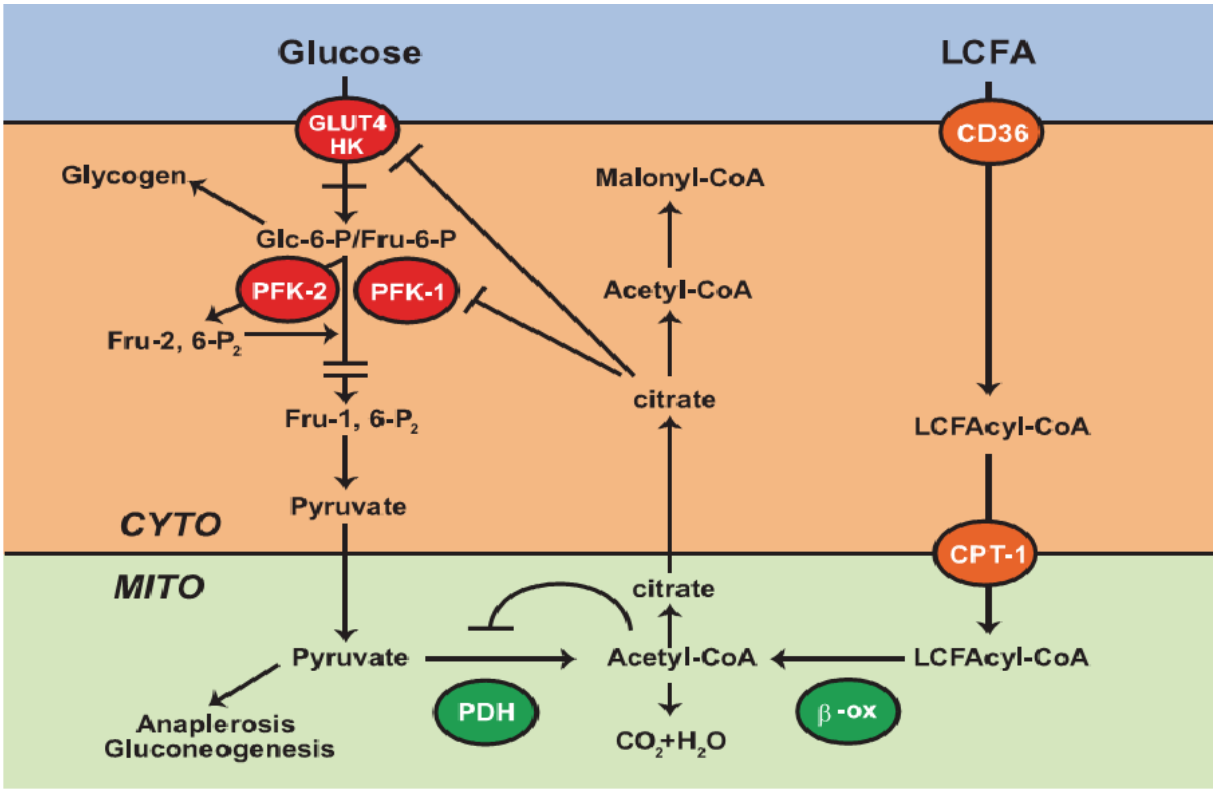


Figure 2: Mechanism of inhibition of glucose utilization by fatty acid oxidation (Hue and Taegtmeyer, 2009).

II.7. Carnitine degradation in the rumen:

Cao et al. (2008) reported that unprotected L-carnitine showed very low rumen bypass efficiency. Most of L-carnitine was lost in rumen. No L-carnitine was detected in abomasum fluid and in feces, and this could be the proof that rumen microflora metabolizes entirely the L-carnitine and nothing escapes the rumen. On other hand, Carlson et al. (2007) observed that carnitine is not completely degraded by ruminal microorganisms.

Carnitine degradation is greater in ruminal fluid from the cow fed the 50% forage than in ruminal fluid from the cow fed the 75% concentrate diet, and was more rapid in ruminal fluid obtained after 2 weeks of adaptation to dietary carnitine supplementation (LaCount et al., 1996).Several mechanisms are known for the degradation of carnitine by micro-organism, as shown in Figure 3 (Bremer, 1983). Extremely high rumen resistance might also hinder the intestinal release and absorption of L-carnitine in cows (Cao et al., 2008).

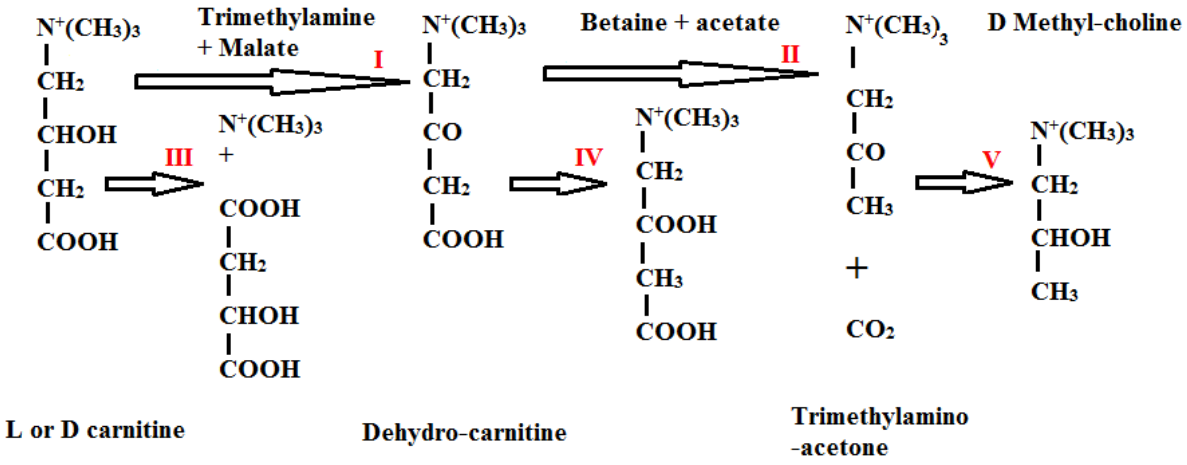


Figure 3: Catabolism of carnitine (Bremer, 1983).

I: Carnitine dehydrogenase, Inducible and specific for D-carnitine and NAD in some micro-organisms. II: this decarboxylation may be spontaneous (non-enzymatic). III: Cleavage in some micro-organisms by unknown enzyme (s). IV: CoA- and ATP-dependent cleavage in some micro-organisms. CoA esters (s) are probable intermediate (s). V: Tri-methyl-amino-acetone is reduced to methyl-choline in some insects and possibly in mammals.

II.8. Effects of L-carnitine supplementation:

Supplementation with carnitine stimulated fatty acid oxidation in vitro (Emery et al., 1992). In dairy cows serum β-hydroxybutyric acid was increased by carnitine infusion in feed-restricted cows(Carlson et al., 2006). In lambs L-carnitine significantly decreased both cholesterol and LDL concentrations in blood (Foroozandeh et al., 2014) in accordance with the results found in dairy cows by LaCount et al., (1996). L-carnitine did not affect feed intake, but improved ether extract digestibility and tended to improve crude protein digestibility in growing lambs (Foroozandeh et al., 2014). In addition, apparent digestibilities of lipid, energy, and total fatty acids increased by carnitine administration (LaCount et al., 1995).In feed-restricted cows, carnitine infusion increased 3.5% fat-corrected milk yield compared with those infused with water (Carlson et al., 2006).Nevertheless, milk yield, milk composition, and DM intake were unaffected by carnitine supplementation, except for increased solid non fatty content (LaCount et al.,

1995). Furthermore, concentrations of glucose, NEFA, and urea N in plasma were unaffected by the amount of dietary carnitine in early lactating cows (LaCount et al., 1996).

HYPOTHESIS AND OBJECTIVES

III. HYPOTHESIS AND OBJECTIVES

III.1. Hypothesis

Heat-stressed dairy goats do not mobilize body fat reserves, despite the fact that they are in negative energy balance. It seems that goats under such conditions degrade their body muscles to keep blood glucose constant. Our hypothesis was that heat-stressed goats are not able to utilize fatty acids as energy source and that L-Carnitine would increase energy efficiency from fatty acids. This would reduce the use of amino acid for glucose production, and thereby results in more available amino acids for milk production.

III.2. Objectives

The objective of this thesis was to study the effects of L-carnitine supplementation to heat-stressed dairy Murciano-Granadina goats on their lactational performance and assess the metabolic changes that might be induced. Some studies have been published on the response of dairy cows to L-carnitine, but as far as we know no studies have been published on the use of L-carnitine in heat-stressed dairy animals.

The following items were evaluated:

- Rumen degradability of L-carnitine products
- Productive variables:
  - Dry matter intake and water consumption
  - Body weight variation and changes in subcutaneous fat.
  - Milk yield and milk composition (fat, protein, lactose, SCC)
- Physiological and metabolic indicators:
  - Body temperature and respiration rate
  - Blood glucose, urea, creatinine, cholesterol, triglycerides, NEFA, BHBA, hematocrit, and hemoglobin
  - Blood Na, K, Cl, iCa, total CO_2, and anion gap

MATERIALS AND METHODS

IV. MATERIALS AND METHODS

IV.1. Animals, Diets and Experimental Design

The experimental work was approved by the Ethical Committee of Animal and Human Experimentation of the Universitat Autonoma de Barcelona (CEEAH reference 11/1430) and carried out according to the codes of recommendations for the welfare of livestock of the Ministry of Agriculture, Food and Environment (MAGRAMA) of Spain.

Six multiparous Murciano-granadina dairy goats (134 ± 2 DIM, 2.48 ± 0.02 L/d milk yield; 46.1± 0.5 kg BW), with healthy and symmetrical udders, were used from the herd of the experimental farm of the Universitat Autonoma de Barcelona. Goats were kept in metabolic cages and used in a replicated 6 × 6 Latin square design with 6 periodsof16 d each (adaptation, d 1 to 11; measurements, d 11 to 16) and allocated to one of 6dietary treatments in a 3 × 2 (diet × ambient) factorial arrangement.

Factors were: 1) Diet: control (**CON**), supplementation with 1 g of L-carnitine from Lohmann (**LOH**; CarnEon20 Rumin-Pro, Lohmann Animal Health, Germany), supplementation with 1 g of L-carnitine from Sintal (**SIN**; Carnitina 20%, Sintal, Vicenza, Italy), and 2) Ambient: thermo-neutral (**TN**; ranging from 15 to 20°C; THI = 59 to 65) or heat stress (**HS**; from 0900 to 2100h at 35°C and from 2100 to 0900h at 28°C with 45±5% relative humidity; THI = 84 and 75, during the day and night, respectively). This resulted in 6 treatment combinations: TN-CON, TN-LOH, TN-SIN, HS-CON, HS-LOH, and HS-SIN.

Goats had a 4 wk pre-experimental period under TN conditions for adapting to diet and metabolic cages. When goats were switched from TN to HS conditions, the temperature increased gradually (1 d at 28°C and 1 d at 30°C), but the change from HS to TN was abrupt.

Data of environmental temperature and humidity were recorded every 10 min throughout the experiment by data loggers (Opus 10, Lufft, Fellbach, Germany). The THI values were calculated according to NRC (1971) as follows:

$$THI = (1.8 \times T_{db} + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T_{db} - 26.8)]$$

Where T_{db} is the dry bulb temperature (ºC) and RH is the relative humidity (%).

The TN goats were kept indoors and room temperature maintained approximately constant with the help of an electric heater equipped with a thermostat (3.5 kW; General Electric, Barcelona, Spain). On average, temperature and relative humidity for the TN goats were 17.4 ± 0.5ºC and 62 ± 5% (THI = 63). The HS goats were kept in a 4 × 6 × 2.3 m climatic chamber (Euroshield, ETS Lindgren-Euroshield Oy, Eura, Finland) provided of automatic temperature and humidity control system (Carel Controls Ibérica, Barcelona, Spain). A continuous 90 m^3/h air turnover was maintained throughout the experiment. Photoperiod was maintained constant throughout the experiment at 12-12 h light- dark (0900 to 2100 h) in both TN and HS groups.

Goats were milked twice daily (0800 and 1700 h) with a portable milking machine set at 42 kPa, 90 pulses/min, and 66% pulsation ratio, provided of recording jars (5 L ± 5%). Milking routine included cluster attachment without udder preparation or teat cleaning, machine milking, machine stripping before cluster removal, and teat dipping in an iodine solution (P3-ioshield, Ecolab Hispano-Portuguesa, Barcelona, Spain).

A total mixed ration was distributed to all goats once daily, after the morning milking, and adjusted at 30% leftover based on the previous day intake. The ration contained(as fed): alfalfa hay 60.4%, ground barley grain 15%,beet pulp 9.1%,ground corn grain 7.5%, soybean meal 3%,sunflower meal 3%,molasses 1%,salt 0.6%, sodium bicarbonate 0.2%,and CVM for goats 0.2%. The chemical composition and nutritive value of the ration are shown in table 2.Mineral and vitamin blocks were freely available for each goat. Doses of carnitine (5 g each that contained 1 g pure L-carnitine) were daily weighed by an electronic scale (Mobba Barcelona, Barcelona, Spain), mixed with 50 g crushed barley, and offered individually before the a.m. milking. The control goats were fed 50 g crushed barley without carnitine supplementation. The chemical composition of carnitine supplements is shown in table 3.

Table 2: Chemical composition and nutritive value (DM basis) of the total mixed ration used for dairy goats.

Item	Total mixed ration
Component, %	
Dry matter	88.2
Organic matter	88.1
Crude protein	15.1
Ether extract	1.58
Neutral detergent fiber	39.3
Acid detergent fiber	28.6
Nutritive value[1]	
UE_m,[2] /kg	0.91
UFL, [3] /kg	0.90
NE_L, Mcal/kg	1.54
PDIE,[4] g/kg	68
PDIN,[5] g/kg	94
PDIA, [6] g/kg	21
Ca, g/kg	9.5
P, g/kg	3.0

[1] Calculated according to the Institut National de la Recherche Agronomique (INRA, 2007). [2] Fill units for sheep (1 UE_m = 1 kg DM of reference grass). [3] Feed units for lactation (1 UF_L = 1.7 Mcal of NE_L). [4] Protein digested in the small intestine supplied by microbial protein from rumen-fermented OM. [5] Protein digested in the small intestine supplied by microbial protein from RDP. [6] Protein digested in the small intestine supplied by RUP.

Table 3: Chemical composition (DM basis) of the L-Carnitine used for supplementation of the dairy goats.

Item	CarnEon 20 Rumin-Pro® (Lohman)	Carnitina 20%® (Sintal)
Component, %		
Dry matter	91.9 ± 0.3	92.6 ± 0.2
Crude protein	10.7 ± 0.1	10.5 ± 0.1
Ether extract	46.7 ± 0.4	45.7± 0.8

IV.2. Evaluation of L-Carnitine Degradability:

The ruminal degradability was determined by the *in situ* technique using nylon bags according to Ørskov and McDonald (1979).One non-lactating dairy cow fitted with a rumen cannula was used. The degradability of LOH was evaluated twice when the cow was fed 100% lucerne hay *ad libitum* (DM, 92.4%; CP, 18.8%;CF, 25.0%;NDF, 35.9%;and ADF,27.1%; DM basis) or 20% lucerne hay and 80% concentrate (barley31.5%, corn41.5%, soybean meal 44 5%, sodium bicarbonate 1%, calcium phosphate 0.4%, calcium carbonate 0.5%, salt 0.7%, premix0.4%; as fed).The degradability of SIN was evaluated only once when the cow was fed the 20% lucerne and 80% concentrate diet. Diet was offered daily in both cases at 0800 h. The nylon bag size was 11 × 5 cm with a pore size of 50 µm (Ankom Technology: Ref. R510). Samples of 5gL-carnitine were put in the nylon bags, which were heat-sealed at1 cm of the end. All bags were inserted in the rumen at 0800 h (just before feeding) and removed at 2, 4, 6, 8, 12, 16, 24, 48 and 72 h of incubation.

After removal from the rumen, the bags were immediately machine washed for 15 min (three cycles of 5 min) using tap water. The 0 h bags were not incubated and only were washed. Microbial decontamination was done by the freezing method as described by Zakraoui et al. (1995). Bags were frozen at −18°C for 24h, thawed at 50°C in a water bath, immersed in 0°C water for 5 min, and finally washed for 10 min in tap water. The bags were dried at 105ºC for 24 h, and then weighed. Three bags were prepared for each L-

carnitine brand (Lohmann and Sintal) at each incubation time point. The average weight of the 3 bags was used to calculate the degradability.

The degradability was obtained from the exponential curve (Ørskov and McDonald, 1979).

$$\mathbf{Y = a + b \cdot (1 - e^{-ct})}$$

Where, Y is the actual degradation at time't', the constant '*a*' represents the soluble and very rapidly degradable component and '*b*' represents the insoluble but potentially degradable component, which is degraded at constant fractional rate (*c*) per time unit.

The effective degradability (ED) expressed as percentage of DM basis, was then estimated by the following equation:

$$\mathbf{ED = a + (b \cdot c)/c + k}$$

Where, *k* is the fractional outflow rate of small particles from the rumen, and a value of 0.06was used (INRA, 1988).

IV.3. Measurements, Sampling and Analyses:

IV.3.1. Body Temperature and Respiration Rate:

Rectal temperatures and respiration rates were recorded at 0800, 1200, and 1700 h. Rectal temperature was measured by a digital clinical thermometer (Model ICO Technology "mini color", Barcelona, Spain; range, 32 to 43.9ºC; accuracy, ± 0.1ºC), whereas visually accounted number of inhalations and exhalations during 60 s indicated the respiration rate.

IV.3.2. Feed Intake and Water Consumption:

Feed intake was recorded daily by an electronic scale (model Fv-60K; A&D Mercury PTY, Thebarthon, Australia; accuracy, ± 20 g) and water consumption was daily measured by an electronic scale (model JC30; JC Compact, Cobos Precision, Barcelona, Spain; accuracy, ± 10g). Trays with saw dust were put below the drinking troughs and weighed once daily to take into account water wastes. Feed samples were collected before the beginning of each experimental period and were ground through a 1 mm stainless steel

screen, and then analyzed for DM, ADF, NDF, and ash according to analytical standard methods (AOAC International, 2003). The Dumas method (AOAC International, 2003) with a Leco analyzer (Leco Corporation, St. Joseph, MI) was used for N determinations and CP was calculated as percentage of N × 6.25.

IV.3.3. Ration particle size measurement:

A sample of 5% total orts was collected daily during the measurement period, and mixed together to make a composite of orts per goat and period. Particle size of the ration and orts were measured according to the technique described by Heinrichs and Kononoff (2002).The Penn State Particle Separator (DSE 2013-186, Pennsylvania, USA) was used and the 4plastic separator boxes were stacked on top of each other in the following order: sieve with the largest holes (upper sieve) on top, the medium-sized holes (middle sieve) next, then the smallest holes (lower sieve), and the solid pan on the bottom. Samples (200 g) were placed on the upper sieve and shaken on a flat surface, in one direction, 5 times and then the separator box was rotated one-quarter turn. Vertical motion during shaking was avoided. This process was repeated 7 times, for a total of 8 sets or 40 shakes, rotating the separator after each set of 5 shakes. See the sieve shaking pattern in figure 4.

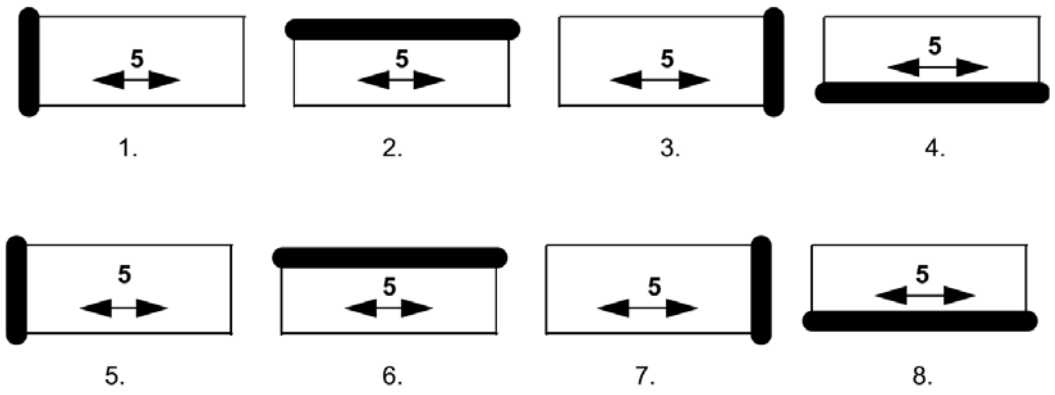


Figure 4: Shaking pattern for particle size separation (Heinrichs and Kononoff, 2002).

The average particle size was calculated using a spreadsheet downloaded from the Penn State Dairy Cattle Nutrition Website (www.das.psu.edu/dcn/catforg/particle).

IV.3.4. Milk Yield and Composition:

Milk yield (kg/d) of individual goats was recorded daily throughout the experiment by the electronic scale used for water consumption measurement. Milk composition was evaluated twice during the measurement period. A milk sample of approximately 100 mL was collected and preserved with an antimicrobial tablet (Bronopol, Broad Spectrum Microtabs II, D&F Control Systems, San Ramon, CA) at 4°C until analysis. Refrigerated milk samples were sent to the Laboratori Interprofessional Lleter de Catalunya (Allic, Cabrils, Barcelona, Spain) for the analyses of TS, fat, protein (N × 6.38), lactose, and SCC using Milkoscan (MilkoScan FT2 - infrared milk analyzer, Foss 260, DK-3400 Hillerød, Denmark) and an automatic cell counter (Fossomatic 5000, Foss Electric, Hillerød, Denmark) previously calibrated for goat milk.

IV.3.5. Body Weight and Subcutaneous Fat Assessment:

In order to measure the body weight variation, goats were weighed at the beginning and the end of each experimental period by a scale (model Tru-Test AG500 Digital Indicator, Auckland, New Zealand; accuracy, ± 20 g).The scale was calibrated by a 5 kg weight before every weighing. The subcutaneous fat thickness was measured by ultrasonography according to Teixeira et al. (2008). The ultrasound images were taken using a VET 180 Plus ultrasound (Sonosonite, Bothell, WA) with a 5-MHz probe (veterinary model). When the ultrasound images were taken at the breast bone, goats were restrained in dorsal recumbency on a table. After clipping, an acoustic gel was used to allow a better contact surface between the probe and the skin. The fat thickness measurement was taken in a perpendicular position to the ventral midline at the level of the third and fourth sternebrae. When a clear image was obtained, it was recorded, digitized, and transferred to a computer for subsequent processing. Measurements were then performed by an image analysis program (ImageJ 1.48v Rasband Wayne National Institute of Health State, available at (imagej.nih.gov/ij/download.html).Three measurements of the distance between the skin and the sternum were done for each goat (Figure 5).

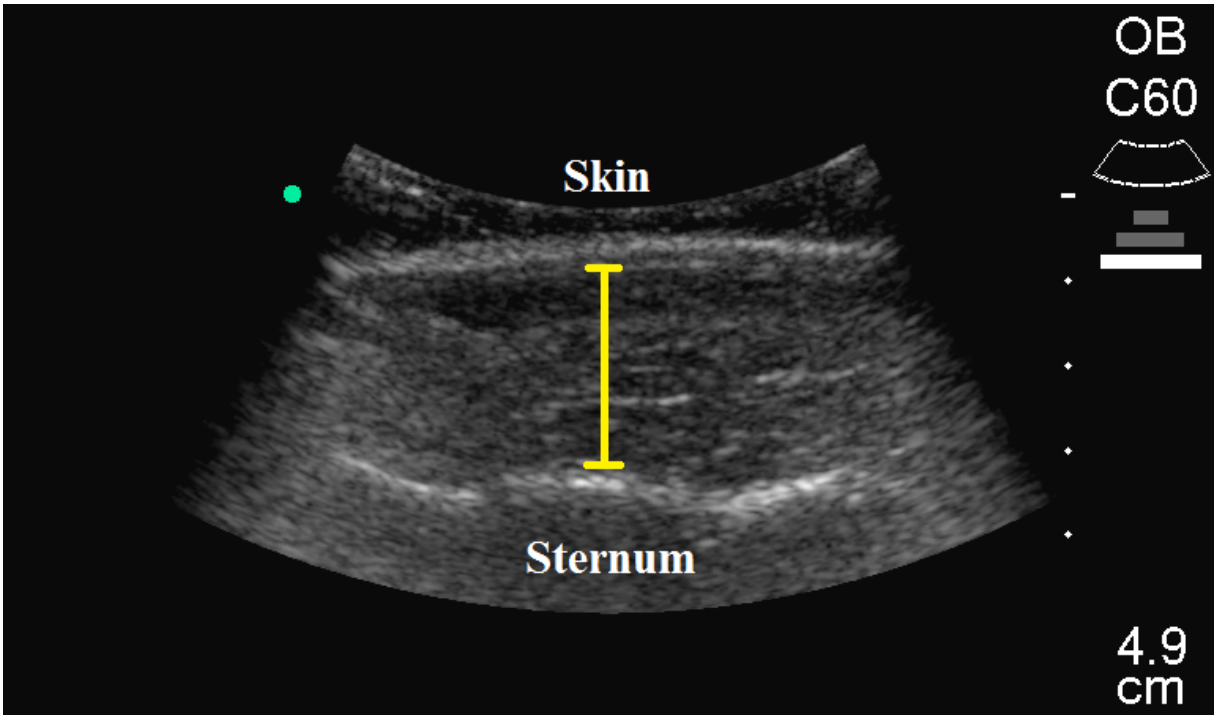


Figure 5: Ultrasound fat thickness measurement in sternum region at the third and fourth sternebrae of a dairy goat.

IV.3.6. Blood Analyses:

Blood samples were taken at the last day of each measurement period from the jugular vein into 10 mL plastic lavender vacutainers with spray-coated K2-EDTA (BD Diagnostics, Franklin Lakes, NJ) before morning feeding. Plasma was obtained by centrifugation of whole blood for 15 min at 1500 × *g*, and stored at –20°C until the analysis of NEFA, BHBA, triglycerides, and cholesterol at the Servei de Bioquímica Clínica Veterinària of the UAB.The NEFA were determined by colorimetric enzymatic test ACS-ACOD method using a commercial kit (Wako Chemicals, Neuss, Germany). The BHBA was determined by kinetic enzymatic method using commercial kit (Ranbut, Randox, UK). Cholesterol was analysed by the enzymatic method (cholesterol esterase/peroxidase), whereas triglycerides were analysed with enzymatic method (glycerol phosphate oxidase) using an Olympus analyser (Olympus AU400, Germany).

Moreover, blood samples (approximately 0.3 mL) were collected by insulin syringes (1 mL; BD Micro-Fine, BD Medical-Diabetes Care, Franklin Lakes, NJ) and immediately

analyzed for major ions and metabolites. A single drop of whole blood was applied to disposable cartridges containing biochemical and silicon chip technology (i-StatChem8+, Abbott Point of Care, Princeton, NJ). Then, the cartridge was inserted into an i-Stat handheld analyzer, and the results of glucose, urea, Cl, Na, K, iCa, total CO_2 concentration, anion gap, hematocrit, hemoglobin, creatinine and base excess were obtained. Blood carnitine fractions (free carnitine, acetyl carnitine and total carnitine) were determined for CON and LOH goats only using a quasi-solid phase extraction without derivatization reactions by means of normal-phase liquid chromatography and electro spray ionization tandem mass spectrometry (Hirche et al., 2009).

IV.4. Statistical Analyses:

Data were analyzed by the PROC MIXED for repeated measurements of SAS version 9.1.3 (SAS Inst. Inc., Cary, NC). The statistical mixed model contained the fixed effects of the temperature (TN, HS), supplementation (CON, LOH, SIN), period; the random effect of the animal; the interactions temperature × supplement, temperature × period, supplement × period, temperature × supplement × period; and the residual error. The model took into account the possible carryover effects of previous HS periods through the temperature× period interaction. For the data of rectal temperature and respiratory rate measured at 0900, 1200 and 1700, a fixed factor of the hour of day was added to the model. Differences between least squares means were determined with the PDIFF test of SAS. Significance was declared at $P < 0.05$and tendency at $P<0.10$unless otherwise indicated.

RESULTS AND DISCUSSION

V. RESULTS AND DISCUSSION:

V.1. L-carnitine Rumen Degradability:

Three L-carnitine samples from LOH (raw without treatment, water-washed, and after 72h of rumen incubation) were examined under microscope (×45). The L-carnitine raw particles were small and greasy white balls having lighter white dots. After washing in water, the balls looked less bright but they kept the spherical structure; the white dots were more discernable. The 72h rumen-incubated sample showed incrusted feed inside the spherical structure of the protected L-carnitine conferring a darker color to the product (Figure 6).

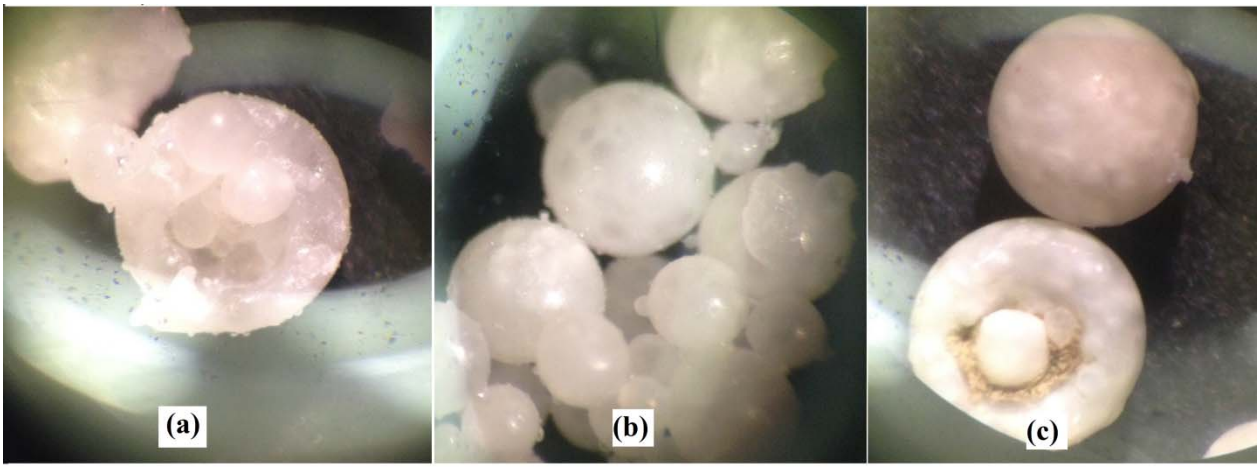


Figure 6: Particles of raw L-carnitine (a), after washing by water (b) and after 72h of rumen incubation (c) viewed under microscope (×45).

Values of DM and CP degradability in the rumen for both L-carnitine products (LOH and SIN) and the two tested diets are shown in table 4 Figure 6 and 7. After 16h of rumen incubation, the effective degradability ($ED_{16\text{-}h}$) of dry matter was similar for the 2 L-carnitine products (72.8 and 78.0 % for LOH and SIN, respectively). Furthermore, no differences were detected between the parameters (a, c) of the equation defining the prediction model of Ørskov and MacDonald (1979) for both L-carnitine products under the same diet conditions (20% forage:80% concentrate). However, LOH carnitine the $ED_{16\text{-}h}$ dry matter degradability (85.8%) increased when the ration shifted from 20% forage to 100% forage. This agrees with the results of LaCount et al. (1996)who reported that carnitine degradation was greater in ruminal fluid from cows fed

the 100% forage diets than in ruminal fluid from cows fed 75% concentrate diets and it was more rapid in ruminal fluid obtained after 2 wk of carnitine supplementation. Constants of the degradation equation differed between the 100 and 20% forage rations, being “c” (the constant fractional rate of degradation per unit of time) greater in the80% concentrate diet.

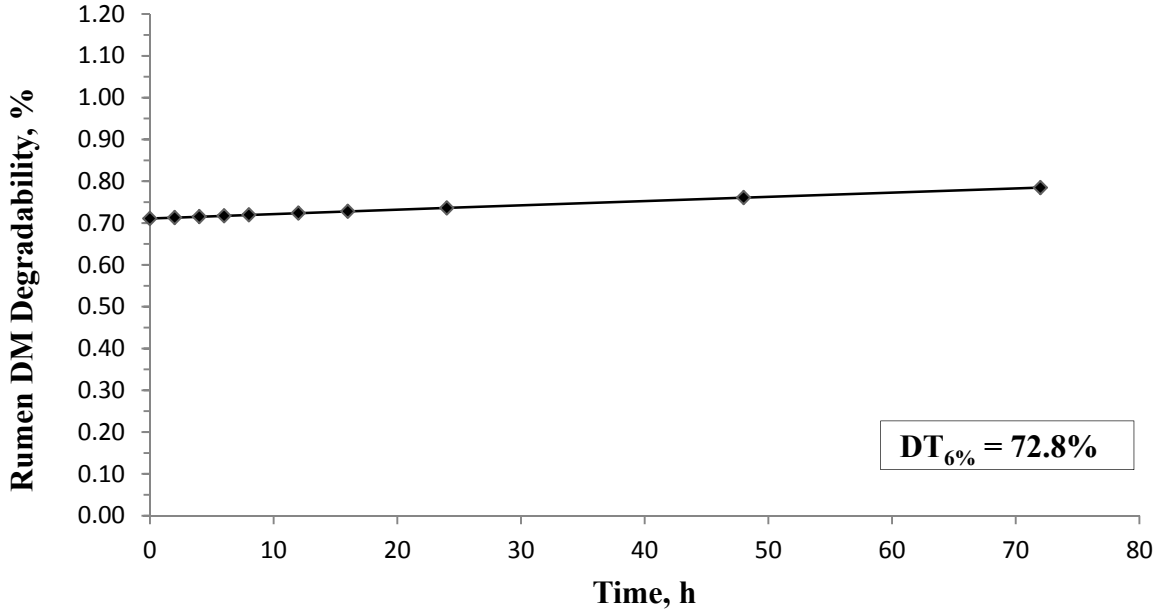
With regard to CP degradability, the $ED_{16\text{-}h}$ values were higher compared to those of DM. The LOH and SIN products showed similar values of $ED_{16\text{-}h}$, under the high concentrate diet, being their values 95.4 and 94.3%, respectively. Additionally, no differences were detected for the 3 parameters (a, b and c) for LOH and SIN under the high concentrate diet. The 100% forage diet showed lower values of $ED_{16\text{-}h}$(85.6%) when compared to the 80% concentrate diet, being the “a” and “b” values also different when the diet was changed.

Table 4: Parameters of the degradability at 16 h for Lohmann (LOH) and Sintal (SIN)L-carnitine products according to 100% hay diet (100 F) and 80% concentrate diet (20 F : 80 C). Parameters a, b, and c were according to the Ørskov and McDonald (1979) model: $Y = a + b \cdot (1 - e^{-ct})$

Items	a[1]	b[2]	c[3]	ED[4], %	TD[5], %	SE[6]
DM						
LOH, 100 F	0.86±0.03[a]	$-8.3\times10^{4}\pm3.2\times10^{4}$[a]	$29.4\times10^{-9}\pm0.1\times10^{-9}$[a]	85.8	-8.0×10^{4}	0.04
LOH, 20 F : 80 C	0.71±0.01[b]	1.00[b]	$1.1\times10^{-3}\pm0.4\times10^{-3}$[a]	72.8	171.1	0.01
SIN, 20 F : 80 C	0.71±0.03[b]	0.08±0.03[b]	$42.7\times10^{35}\pm0.1\times10^{35}$[b]	78.0	78.0	0.01
CP						
LOH, 100 F	0.51±0.05[a]	0.42±0.06[a]	0.29±0.09	85.6	92.8	0.02
LOH, 20 F : 80 C	0.85±0.04[b]	0.12±0.04[b]	0.43±0.30	95.4	96.9	0.01
SIN, 20 F : 80 C	0.80±0.03[b]	0.18±0.03[b]	0.25±0.10	94.3	97.8	0.01

[1] Soluble and very rapidly degradable component.[2] Insoluble but potentially degradable component.[3] Constant fractional rate of degradation per unit time.[4]Effective degradability.[5] Total degradability.[6]Sum of the squares of residuals (deviations of predicted from actual empirical values of data). [a-b] Values within a column for CP and DM with different superscripts differ (P <0.05).

1)



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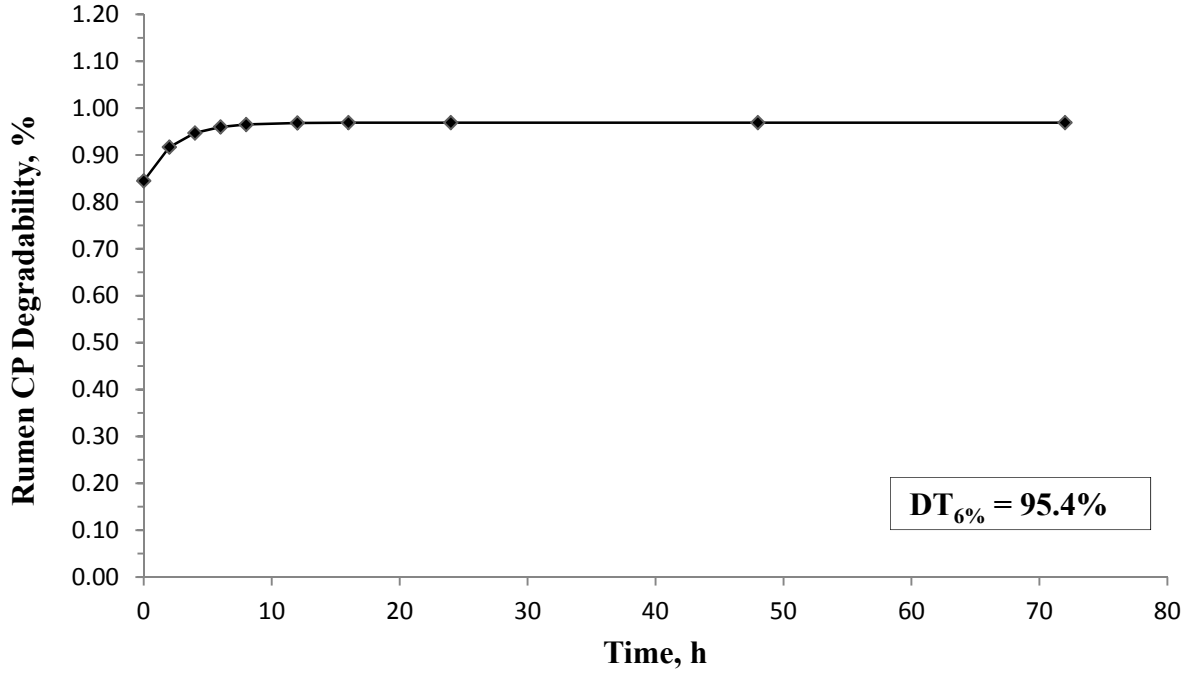
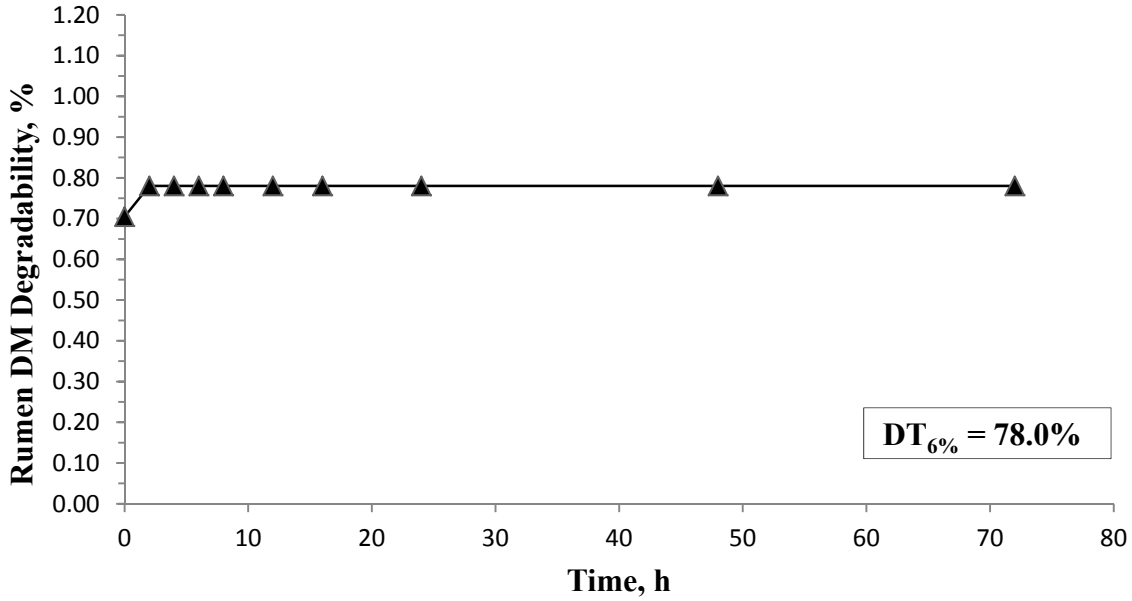


Figure 7: Lohmann L-carnitine rumen degradability of dry matter (1) and crude protein (2) under 20% forage: 80% concentrate conditions.

1)



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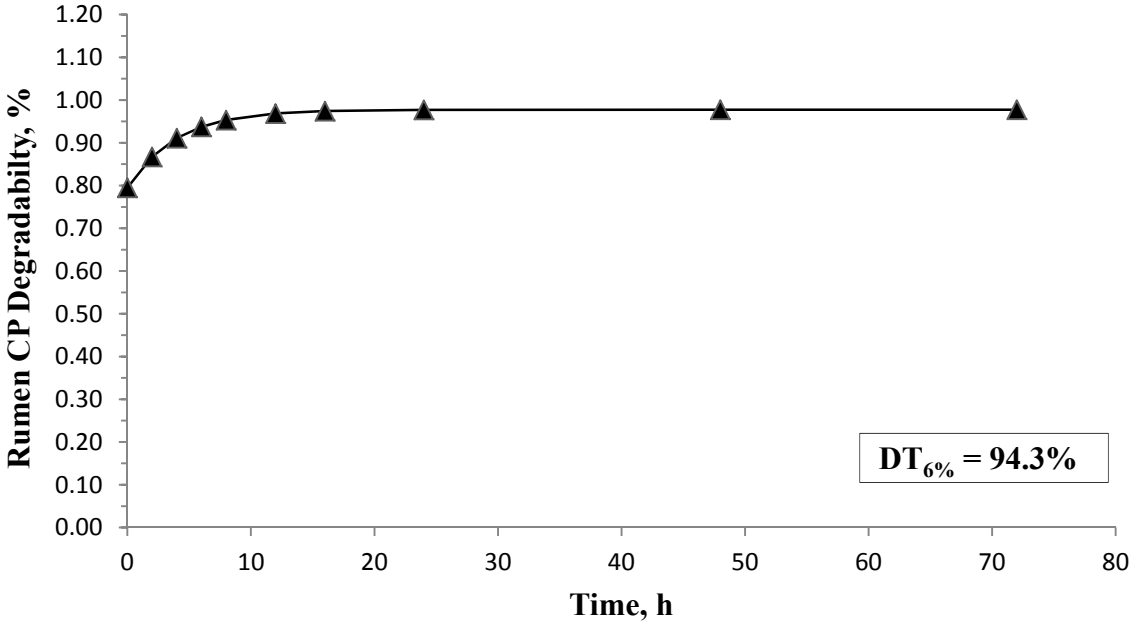

Figure 8: Sintal L-carnitine rumen degradability of dry matter (1) and crude protein (2) under 20% forage : 80% concentrate conditions.

V.2. Body Temperature and Respiration Rate:

Rectal temperatures and respiration rates data at 0800, 1200 and 1700 h are shown in table 5. The HS goats showed a greater ($P< 0.01$) rectal temperatures and respiration rates than TN goats. This agrees with the results of Sivakumar et al. (2010), Hamzaoui et al. (2013), and Dangi et al. (2014)where goats exposed to heat stress had high rectal temperatures and respiratory rates. The increment in respiration rate under HS conditions is a known mechanism for dissipating heat load by evaporation. The supplementation with L-carnitine had no effect on rectal temperature or respiratory rate.

Table 5: Respiratory rate and body temperature of Murciano-Granadina dairy goats under thermal neutral (TN) and heat stress (HS) conditions. In each ambient conditions, goats were fed a control diet (CON) or supplemented with 1g L-carnitine from Lohmann (LOH) or Sintal (SIN).

Item	TN			HS			SEM	Effect ($P<$)		
	CON	LOH	SIN	CON	LOH	SIN		Temp	Carn	T×C
Body temperature, ºC								0.01	0.51	0.73
0800h	38.5	38.5	38.4	39.1	39.3	39.2	0.07			
1200h	38.6	38.5	38.5	39.6	39.7	39.5	0.07			
1700h	38.8	38.6	38.7	39.9	39.8	39.7	0.07			
Respiration rate, breaths/min								0.01	0.88	0.81
0800h	36	34	36	80	90	79	3			
1200h	36	35	37	118	110	115	3			
1700h	38	38	38	123	121	117	3			

V.3. Feed and Water Intakes:

On average, DMI decreased in HS animals by 26% throughout the experimental period (1.90vs. 2.58kg/d; $P<0.001$; table 6), which agrees with the results previously observed in dairy goats (Hamzaoui et al., 2013). On the other hand, L-carnitine supplementation did not affect feed intake, which is in accordance with the results found by Chapa et al. (2001) in growing sheep

Nevertheless, Foroozandeh et al. (2014) found that DMI decreased when 100 g/dL-carnitine were supplemented in sheep.

Heat-stressed goats tended to ($P< 0.06$) to consume larger particle size food compared to TN goats, as indicated by the average particle size of their orts (6.2 vs. 4.5 mm for TN and HS, respectively; table 6). Castro-Costa et al. (2014) reported that HS decreases rumen pH by itself in dairy goats, and it is possible that HS-goats in the current study ate larger particles to alleviate the decreased ruminal pH. No effect of L-carnitine supplementation on the orts particle size was observed.

Results of feed intake and water consumption throughout the experiment are shown in Table X. The HS goats showed a tendency to have greater water consumption compared to TN goats (6.67vs. 5.79L/d; $P<0.12$), which agrees with the find of Hamzaoui et al. (2013)who reported greater water consumption and water evaporation in HS Murciano-Granadina dairy goats. Increased water intake in HS goats was mainly used for boosting heat loss by evaporation from the skin (sweating) and by respiration (panting). No differences in water consumption were observed in L-carnitine supplemented goats.

Table 6: Feed intake, water consumption and averaged particle size of the orts of thermo neutral (TN) or heat stressed (HS) dairy goats fed diets supplemented or not (CON) with L-carnitine from Lohmann (LOH) or Sintal (SIN).

Item	TN			HS			SEM	Effect ($P<$)		
	CON	LOH	SIN	CON	LOH	SIN		Temp	Carn	T×C
Intake, kg/d										
Feed	2.60	2.56	2.58	1.85	1.95	1.89	0.16	0.01	0.98	0.90
Water	5.66	5.31	6.39	6.49	6.57	6.96	0.73	0.12	0.53	0.87
APS[1], mm	5.3	7.9	5.5	4.0	5.0	4.3	1.1	0.06	0.24	0.67

[1]APS: Average particle size of the orts.

V.4. Milk Yield and Composition

As shown in table 7, milk yield tended ($P < 0.06$) to decrease by 11% in goats exposed to HS (1.63 kg/d) compared to TN goats (1.84 kg/d). This reduction was similar to what observed by Hamzaoui (2014) in HS-dairy goats at mid lactation. The consequences of high ambient temperature in lactating dairy ruminants are well known (West, 2003; Baumgard and Rhoads, 2013; Salama et al., 2014) which include reduced DMI and increased body temperature and, consequently, impaired milk yield and composition. Milk composition was also affected by high ambient temperature; the HS-goats producing lower fat, protein and lactose than TN-goats, similarly as reported by Hamzaoui et al. (2013) who observed that milk of HS-goats contained −12.5% protein than TN-goats.

We hypothesized that L-carnitine supplementation would improve the efficiency of energy use, which would reflect in reducing the adverse effect of HS on milk production. However, L-carnitine supplementation did not affect neither milk yield nor milk composition, which agrees with findings of LaCount et al. (1995) in dairy cows. Nevertheless, feed-restricted dairy cows with abomasum infused L-carnitine produced greater 3.5% FCM than the control group which was infused with water (Carlson et al., 2006).

V.5. Body Weight and Subcutaneous Fat Assessment

Changes in body weight and subcutaneous fat were expressed as the difference between the values at the start and the end of each experimental period (table 8). On average, the HS-goats lost 146 g/d of BW, whereas TN goats gained 139 g/d, agreeing with the results of Hamzaoui et al. (2013). A portion of the BW changes of TN and HS goats included the inevitable variations in the digestive tract content, which were unknown in our data. Supplementation with L-carnitine affected the overall BW variation per period. This agrees with the results found in growing sheep in which L-carnitine did not affect average daily gain (Chapa et al., 2001).

The values of ultrasound fat measurements revealed that HS goats lost subcutaneous fat thickness compared to TN (−0.72vs. +0.64 mm; $P < 0.05$). L-carnitine supplementation did not

Table 7: Milk yield and milk composition of thermoneutral (TN) or heat stressed (HS) dairy goats fed diets supplemented or not (CON) with L-carnitine from Lohmann (LOH) or Sintal (SIN).

Item	TN			HS			SEM	Effect (P <)		
	CON	LOH	SIN	CON	LOH	SIN		Temp	Carn	T×C
Milk Yield, kg/d	1.90	1.80	1.81	1.59	1.69	1.62	0.14	0.06	0.96	0.73
FCM 3.5%, L/d[1]	2.28	2.15	2.11	1.81	1.90	1.82	0.19	0.03	0.88	0.80
Milk Composition, %										
Total solids	8.89	8.91	8.89	8.37	8.46	8.49	0.19	0.01	0.93	0.95
Fat	4.33	4.21	4.15	4.02	3.96	3.90	0.20	0.08	0.73	0.98
Protein	3.51	3.54	3.55	3.14	3.22	3.23	0.20	0.05	0.95	0.99
Lactose	4.64	4.65	4.61	4.47	4.47	4.50	0.06	0.01	0.99	0.79
Fat yield, g/d	85.8	80.7	78.3	66.5	69.3	66.0	7.8	0.02	0.86	0.84
Protein yield, g/d	69.1	64.6	66.4	50.3	54.5	53.7	6.0	0.01	0.99	0.74
Log SCC[2]	5.97	6.00	5.99	6.30	6.22	6.05	0.23	0.28	0.87	0.84

[1]Fat corrected milk at 3.5%; FCM = L × [0.432 + 0.162 × (fat %)], being L liters of milk yield. [2]Log SCC: logarithm of somatic cell count.

affect subcutaneous fat mobilization. In addition, BW variation and ultrasound measurements showed positive correlation (r = 0.54), demonstrating that HS goats in negative energy balance would mobilize fat without being detected by blood indicators of fat mobilization as NEFA and BHB (see below; Baumgard and Rhoads, 2013; Salama et al., 2014). Mendizabal et al. (2007; 2010) indicated that, for the processes of storage and mobilization of fat reserves in adult goats, the subcutaneous fat was particularly active and appears to be highly specialized for lipid accumulation and mobilization.

Table 8: Body weight variation and subcutaneous (SC) fat variation assessed by ultrasonography of thermoneutral (TN) or heat stressed (HS) dairy goats fed diets supplemented or not (CON) with L-carnitine from Lohmann (LOH) or Sintal (SIN).

Item	TN			HS			SEM	Effect (*P*<)		
	CON	LOH	SIN	CON	LOH	SIN		Temp	Carn	T×C
BW change, kg	2.25	2.53	1.87	−1.80	−2.42	−2.78	0.94	0.01	0.75	0.89
SC fat, mm	0.09	1.34	0.50	−0.73	−0.65	−0.79	0.70	0.02	0.63	0.71

V.6. Blood L-carnitine

Concentrations of blood L-carnitine fractions are shown in the table 9. The three fractions of L-carnitine were almost triplicated (*P*< 0.001) by LOH supplementation in both TN and HS groups, despite the high rumen degradation observed. LaCount et al. (1995) reported that carnitine concentrations in plasma and liver increased when L-carnitine was administered into either the rumen or abomasum, indicating that both sites of administration were equally effective for increasing carnitine concentrations in tissues. Furthermore, LaCount et al. (1996) reported a linear increase in plasma and milk carnitine concentrations as dietary carnitine increased. Chapa et al. (2001) reported also an increased plasma total L-carnitine in supplemented growing sheep. Free L-carnitine represented approximately 75% of total L-carnitine. Basal levels of free L-

carnitine observed in our goats (19.4 μmol/L) were lower than those reported in Arabian camels (71.3 μmol/L; Alhomida et al., 1995) and Holstein dairy cows (63.5 μmol/L; LaCount et al., 1995). Free L-carnitine concentrations numerically decreased ($P< 0.13$) by HS (−14% on average) and it seems that this free carnitine was transformed to acetyl-carnitine (HS groups had +16% acetyl-carnitine). However, it is not clear in what metabolic process (es) this extra acetyl-carnitine was used as we did not detect any significant difference in the measured variables.

Table 9: L-carnitine concentration in plasma of thermoneutral (TN) or heat stressed (HS) dairy goats fed diets supplemented or not (CON) with L-carnitine from Lohmann (LOH) or Sintal (SIN).

L-Carnitine,	TN		HS		SEM	Effect ($P<$)		
μmol/L	CON	LOH	CON	LOH		Temp	Carn	T×C
Free	20.42	61.23	18.42	50.50	4.06	0.13	0.01	0.30
Acetyl-carnitine	6.79	17.49	6.90	21.24	2.08	0.35	0.01	0.38
Total	26.78	78.30	24.90	71.30	4.32	0.32	0.01	0.56

V.7. Main Metabolites in Blood

Blood glucose, urea, creatinine, hematocrit and hemoglobin of TN and HS goats supplemented or not with L-carnitine are shown in table 10. Neither ambient temperature nor L-carnitine supplementation affected most of blood metabolites. The decrease ($P< 0.01$) in blood urea concentration by HS could be explained by their lower DM intake and, consequently, reduced N intake. Despite the reduced feed intake, HS goats were able to keep similar glucose levels to TN goats. Creatinine levels increased ($P< 0.01$) by HS, which might indicate increased muscle degradation. It is possible that the amino acids produced from muscle degradation were used for gluconeogenesis resulting in increased production of glucose.

Values of total blood CO_2 were lower in HS compared to TN goats, which agrees with previous studies (Sivakumar et al., 2010; Hamzaoui et al., 2013). The greater respiration rate

observed in HS goats contributed to a greater loss of CO_2. The Cl^- and K^+ blood concentrations were greater ($P< 0.05$) in HS than in TN goats. Collier et al. (2006)reportedthat HS dairy cows increased K requirements by as much as 12% because cattle sweat is high in K. The blood iCa concentration also tended ($P< 0.10$) to be greater in HS (1.29 mmol/L) compared to TN goats (1.26 mmol/L).

Table 10: Blood indicators of thermoneutral (TN) or heat stressed (HS) dairy goats fed diets supplemented or not (CON) with L-carnitine from Lohmann (LOH) or Sintal (SIN).

Item	TN			HS			SEM	Effect (*P*<)		
	CON	LOH	SAN	CON	LOH	SAN		Temp	Carn	T×C
Na, mmol/L	148.0	147.6	147.0	147.0	147.0	147.5	0.52	0.35	0.88	0.30
K, mmol/L	3.47	3.47	3.59	3.84	3.87	3.56	0.15	0.04	0.75	0.24
iCa, mmol/L	1.28	1.27	1.23	1.27	1.31	1.29	0.02	0.07	0.38	0.31
Cl, mmol/L	103.8	103.6	101.8	107.9	107.8	107.3	0.67	0.01	0.10	0.50
TCO_2,mmol/L	25.69	25.86	26.53	20.86	21.19	21.19	0.7	0.01	0.68	0.87
AnGap	23	22.67	23.5	22.83	23	23.83	0.54	0.71	0.25	0.87
Glucose, mg/dL	59.08	59.58	58.75	59.92	58.58	60.25	1.71	0.74	0.96	0.73
Urea, mg/dL	23.16	24.49	23.66	18.33	19.83	18.16	1.85	0.01	0.71	0.97
Creatinine,	0.47	0.52	0.49	0.57	0.54	0.56	0.02	0.01	0.91	0.20
Hematocrit, % PCV	17.55	18.55	18.05	17.72	18.39	17.39	0.92	0.77	0.61	0.90
Hemoglobin, g/dL	5.98	6.31	6.15	6.05	6.26	5.91	0.31	0.78	0.61	0.89

V.7.1. Fat metabolism indicators

Supplementation with L-carnitine did not affect blood NEFA, BHBA, cholesterol or triglycerides concentrations (table 11). Dairy cows in early lactation have decreased blood NEFA and cholesterol concentrations when supplemented with 10g/d of protected carnitine (Scholz et al., 2014).In addition, Citil et al. (2009) reported that the oral administration of 500 mg/50kg BW of L-carnitine decreased serum triglyceride and cholesterol. Nevertheless, and agreeing with our findings, Carlson et al. (2006) reported that plasma NEFA was not altered by L-carnitine abomasal infusion in mid lactating dairy cows.

As indicated above, SC fat thickness measured by ultrasonography decreased by HS, indicating that goats would have mobilized body fat reserves. However, both blood NEFA and BHB did not change by HS in the current study and previous studies done in dairy goats (Hamzaoui et al., 2013) and dairy cows (Baumgard and Rhoads, 2013). This might indicate that really there was mobilization but NEFA were rapidly taken up by the mammary gland for fat synthesis and were not transformed to ketone bodies by the liver.

Table 11: Fat metabolism indicators in the plasma of thermoneutral (TN) or heat stressed (HS) dairy goats fed diets supplemented or not (CON) with L-carnitine from Lohmann (LOH) or Sintal (SIN).

Metabolic Indicator	TN			HS			SEM	Effect (*P*<)		
	CON	LOH	SIN	CON	LOH	SIN		Temp	Carn	T×C
Triglycerides, mg/dL	17.6	18.0	17.5	17.1	17.1	19.4	1.3	0.88	0.66	0.50
Cholesterol, mg/dL	74.7	76.2	76.6	85.5	79.9	79.3	5.8	0.22	0.91	0.73
NEFA, mmol/L	0.08	0.13	0.09	0.15	0.12	0.09	0.02	0.29	0.33	0.28
BHBA, mmol/L	0.72	0.70	0.72	0.80	0.85	0.82	0.11	0.19	0.97	0.93

VI. CONCLUSION

VI. CONCLUSIONS

General:

The present study evaluates the use of rumen-protected L-carnitine products in dairy goats under thermo-neutral and heat stress conditions. Heat stress affected negatively the lactational performances of dairy goats. Although micro encapsulated L-carnitine was apparently degraded in the rumen, it was really absorbed (high levels of blood carnitine fractions in supplemented animals), but it did not significantly change the productive and metabolic parameters measured in the current study.

Specific:

- Both micro encapsulated L-carnitine products (Lohman and Sintal) showed similar chemical composition and a high degradability in the rumen under *in situ* conditions. The degradability of the protected L-carnitine varied according to the type of diet fed to the animal.
- Despite the high rumen degradability, the concentrations of free- and acetyl-carnitine in blood were much greater in the supplemented animals than in the control. There was an indication that heat-stressed goats used the L-carnitine, as they had numerically lower free-carnitine that apparently they transformed to acetyl-carnitine. However, it is not clear in what metabolic process(es) this carnitine was used.
- Typical effects of heat stress were observed in the lactating dairy goats used: greater respiratory rate and body temperature reduced feed intake, lower milk yield and lower milk protein content.
- Heat-stressed goats tended to eat the dietary particles of a larger size, which might be a mechanism to maintain their rumen pH, as heat stress has been demonstrated to cause low rumen pH.
- Based on the measurement of blood non esterified fatty acids and ketone bodies, most of scientific studies propose that heat-stressed dairy animals do not mobilize body fat reserves. Herein we also observed that heat stress did not change blood non

esterified fatty acids and ketone bodies, but we observed that subcutaneous fat thickness decreased by heat stress.

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VII. REFERENCES

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