Thesis of master degree

Nutritional strategies to alleviate the effect of heat stress on Iberian pigs

Author: Salma Al Asmar

Director: Dr. Antoni DALMAU

Facultad de veterinaria

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Abstract

High ambient temperature exposure can cause important consequences on animals' health and production. As pigs have unfunctional sweat glands, they are more sensible to heat stress than other animals. For this reason, this experiment was held in order to study a nutritional strategy to alleviate the effect of heat stress in pigs. The nutritional strategy was to replace 5% of starch by 5% of high oleic sunflower oil in the diet as fat is an important source of energy and lead to a lesser heat increment than carbohydrates and protein.

Therefore, a total of seventy-two castrated males crossed (50% Iberian – 50% Duroc) Iberian pigs weighing an average of 51.1 ± 0.75 Kg were exposed to high ambient temperature of (30 – 32^{0}C) during 5 hours of the day and during the other hours, the temperature was maintained at a minimum of 25°C. The pigs were distributed in three rooms at the rate of four pens per room and six animals per pen. Two treatments were applied based in two different diet composition: control diet (control) and high fat diet (alternative). In addition, two forms of feeding were used. Half of the animals were fed ad-libitum and the other half restricted. Samples for the analysis of hemogram and biochemical were taken at the beginning and at the end of the study and an analysis of meat and carcass quality was performed after slaughter. Moreover, performance (feed intake, body weight) was regularly assessed and pigs' behavior observed daily.

During the experimental period, the feed intake was reduced while there was no difference on the average daily gain, consequently feed conversion ratio was lower in the alternative diet (high fat content) than in the control diet. LDL levels, the bad cholesterol, did not show any difference between the two diets showing that increasing the diet content in fat did not show bad consequences on serum lipid content in pigs. Additionally, feeding the alternative diet resulted in a higher dressing percentage and oleic content in fat. Moreover, the lying and excreting behavior in addition to the social interactions were affected by the temperature and differed between treatments.

In conclusion, replacing the starch by fat during high ambient temperature conditions improved the feed efficiency, carcass characteristics and the final product obtained was more attractive to the consumers due to the higher content in oleic acid.

Key words: Iberian pig, growing, finishing, heat stress, high fat diet, performance, oleic content.

Resumen

La elevada exposición a la temperatura ambiente puede causar importantes consecuencias en la salud y la producción de los animales. Como los cerdos tienen glándulas sudoríparas no funcionales, son más sensibles al estrés por calor que otros animales. Por esta razón, este experimento se realizó con el fin de estudiar una estrategia nutricional para aliviar el efecto del estrés por calor en los cerdos. La estrategia nutricional fue reemplazar el 5% de almidón por 5% de aceite de girasol de alto contenido oleico en la dieta ya que la grasa es una fuente importante de energía y conduce a un menor incremento de calor que los carbohidratos y las proteínas.

Por lo tanto, un total de setenta y dos machos castrados cruzados (50% ibéricos - 50% Duroc) cerdos ibéricos con un promedio de 51,1 ± 0,75 Kg fueron expuestos a altas temperaturas ambientales de (30 - 32^{0}C) durante 5 horas del día y durante las otras horas, se mantuvo la temperatura a un mínimo de 25ºC. Los cerdos fueron distribuidos en tres habitaciones a razón de cuatro corrales por sala y seis animales por corral. Se aplicaron dos tratamientos basados en dos diferentes tipos de dieta: dieta control (control) y dieta alta en grasa (alternativa). Además, se utilizaron dos formas de alimentación. La mitad de los animales fueron alimentados ad-libitum y la otra mitad restringida. Las muestras para el análisis de hemograma y bioquímico se tomaron al inicio y al final del estudio y se realizó un análisis de la calidad de la carne y la canal después del sacrificio. Además, el rendimiento (consumo de alimento, peso corporal) se evaluó regularmente y el comportamiento de los cerdos se observó diariamente.

Durante el período experimental, la ingesta de alimento se redujo, mientras que no hubo diferencia en la ganancia media diaria, por lo tanto, la conversión alimenticia fue menor en la dieta alternativa (alto contenido de grasa) que en la dieta de control. Los niveles de LDL, el colesterol malo, no mostraron ninguna diferencia entre las dos dietas que muestran que el aumento del contenido de la dieta en la grasa no mostró malas consecuencias en el contenido de lípidos séricos en los cerdos. Además, la alimentación de la dieta alternativa dio lugar a un mayor rendimiento de canal y contenido oleico en la grasa. Por otra parte, el comportamiento de dormir (tumbarse) y excreción además de las interacciones sociales se vieron afectados por la temperatura y diferían entre los tratamientos.

En conclusión, el reemplazo del almidón por la grasa durante condiciones de alta temperatura ambiente mejoró la eficiencia de la alimentación, las características de la canal y el producto final obtenido fue más atractivo para los consumidores debido al mayor contenido en ácido oleico.

Palabras clave: Cerdo ibérico, crecimiento, acabo, estrés por calor, dieta alta en grasa, rendimiento, contenido en oleico.

Résumé

L'exposition élevée à la température ambiante peut entraîner des conséquences importantes sur la santé et la production des animaux. Comme les porcs ont des glandes sudoripares non fonctionnelles, ils sont plus sensibles au stress thermique que les autres animaux. Pour cette raison, cette expérience a été réalisée afin d'étudier une stratégie nutritionnelle pour atténuer l'effet du stress thermique chez les porcs. La stratégie nutritionnelle consistait à remplacer 5% d'amidon par 5% d'huile de tournesol à haute teneur en acide oléique, car la graisse est une source importante d'énergie et entraîne une augmentation de la chaleur inférieure à celle des glucides et des protéines.

Par conséquent, un total de soixante-douze mâles castrés (50% de porcs ibériques - 50% Duroc) de porc Ibérique pesant une moyenne de 51,1 ± 0,75 Kg ont été exposés à une température ambiante élevée (30 - 32°C) pendant 5 heures de la journée et pendant Les autres heures, la température a été maintenue à un minimum de 25 ° C. Les cochons ont été distribués dans trois salles au taux de quatre stylos par salle et six animaux par stylo. Deux traitements ont été appliqués à base de deux variétés différentes : régime alimentaire contrôle (témoin) et régime alternatif (gras élevé). De plus, deux formes d'alimentation ont été utilisées. La moitié des animaux ont été nourris ad-libitum et l'autre moitié a été restreinte. Des échantillons pour l'analyse de l'hémogramme et des biochimiques ont été prélevés au début et à la fin de l'étude et une analyse de la qualité de la viande et de la carcasse a été effectuée après l'abattage. Par ailleurs, la performance (consommation d'aliments, poids corporel) a été régulièrement évaluée et le comportement des porcs a été observé quotidiennement.

Au cours de la période expérimentale, la consommation alimentaire a été réduit alors qu'il n'y avait pas de différence sur le gain quotidien moyen, par conséquent, le taux de conversion des aliments était plus faible dans le régime alternatif (teneur élevée en matières grasses) que dans le régime témoin. Les niveaux de LDL, le mauvais cholestérol, n'ont pas montré de différence entre les deux régimes montrant que l'augmentation du contenu alimentaire dans la graisse n'a pas montré de mauvaises conséquences sur la teneur en lipides sériques chez les porcs. En outre, l'alimentation du régime alimentaire alternatif a entraîné un rendement de carcasse et une teneur en acide oléique dans la graisse plus élevés. En outre, le comportement de dormir et d'excrétion en plus des interactions sociales a été affecté par la température et différait entre les traitements.

En conclusion, le remplacement de l'amidon par la graisse lors de conditions de température ambiante élevées a amélioré l'efficacité de l'alimentation, les caractéristiques de la carcasse et le produit final obtenu étaient plus attrayants pour les consommateurs en raison de la teneur plus élevée en acide oléique.
Mots clés: Porc ibérique, croissance, finissement, stress thermique, alimentation à forte teneur en matière grasse, performance, teneur en acide oléique.

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List of abbreviations

ACTH	Adrenocorticotropic Hormone
ADG	Average Daily Gain
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
Ca	Calcium
Cl	Chlorine
CYST	Cysteine
DE	Digestible Energy
ECuLD	Electrical Conductivity at Longissimus Dorsi
ECuSM	Electrical Conductivity at Semimembranosus Muscle
FA	Fatty Acids
FCR	Feed Conversion Ratio
FI	Feed Intake
HDL	High Density Lipoprotein
HS	Heat Stress
IU	International Unit
LDL	Low Density Lipoprotein
LI	Linoleic Acid
LYS	Lysine
ME	Metabolizable Energy
MET	Methionine
MUFA	Monounsaturated Fatty Acids
Na	Sodium

NE	Net Energy
OL	Oleic Acid
P	Phosphorus
PHuLD	PH at Longissimus Dorsi
PHuSM	PH at Semimembranosus Muscle
PUFA	Polyunsaturated Fatty Acids
R SATUR	Rate of Saturated Fatty acids
R UNSAT	Rate of Unsaturated Fatty Acids
SFA	Saturated Fatty Acids
SM	Semimembranosus Muscle
SW	Swine
THR	Threonine
TRP	Tryptophan
UNS/SAT	Unsaturated/Saturated fatty acids
VIT	Vitamin

Introduction

Production efficiency has a great importance in the swine industry where rapid improvements in the lean growth of market pigs and reproductive efficiency were achieved over the past several decades. However, despite the intense genetic selection, pigs still miss functional sweat glands and have a thicker layer of subcutaneous adipose tissue acting as an effective insulation layer, which makes pigs particularly sensitive to high ambient temperature (Brown-Brandl et al., 2004). As heat stress impairs efficiency at every stage of the production cycle, it will represent a future hurdle if the actual production traits are conserved (Brown-Brandl et al., 2004) causing important economic losses (St-Pierre et al., 2003).

During high ambient temperature, heat stressed pigs reduce their voluntary feed intake in order to reduce the metabolic heat production and maintain the homeothermy leading to a depressed growth performance (Le Dividich et al., 1998). In addition, certain physical activities are increased such as hyperventilation. Consequently, the strategies adopted are of high-energy cost that lead to higher maintenance requirements (Kouba et al., 2001).

In addition, pigs are known as social animals having important physical interactions. Normally, the pigs are penned in groups where competition at the feeder and social stress may occur. These factors along with the environmental temperature can affect the feeding behavior and production parameters of the pigs. It was seen that during a heat load, pigs were lying laterally in a higher rate than sternly and avoid physical contact (Geers et al., 1986). Although pigs are known to separate their dunging area from the sleeping area (Fritschen., 1975), they tend to sleep in their excrement and adapt a wallowing behavior in order to cover their body of a cooler layer and consequently cool themselves (Huynh et al., 2005).

According de Coffey (1999), pigs consume feed in order to meet their energy requirements. As pigs reduce their feed intake in order to reduce the metabolic heat production during high ambient temperature conditions, it was seen that supplementing fat to the diet can provide the pigs their energy requirements with a minimum level of heat increment as fat is a high-energy ingredient.

Moreover, it was described that the ad-libitum fed Iberian x Duroc pigs, an excessive body weight is reached at the slaughter age with economic disadvantages because of the increased fat content. For this reason, the feed restriction is applied in order to reduce the animal´s growth and fat deposition (Viguera et al., 2012). However, as there is a close relationship between feed intake and the animal body composition, this strategy can represent an influential factor for the final quality of meat (Ruiz et al., 2002).

The production of the Iberian pigs has a high importance due to the high oleic acid content in their tissues and high concentration of myoglobin and iron (Ventanas et al., 2001). However, the production of "animales de bellota" is and will be insufficient to cover the actual and predictable market demand because of to the limited production area of acorns (Aeceriber, 2007). For this reason, Iberian pigs are reared indoors and fed concentrated-based diets. Moreover, to improve the reproductive performance and the feed efficiency, Iberian pigs are crossed with Duroc.

Therefore, the objective of this study was to determine the effect of replacing 5% of starch by 5% of high oleic sunflower oil in the diet provided ad-libitum or restricted to the Iberian x Duroc pigs subjected to a chronic heat stress during the growing-finishing phase compared with a no added fat diet on the growth performance, carcass characteristics, fatty acid composition and pigs' behavior.

I. Literature review

1. Stress definition

Moberg and Mench (2000) defined stress as "*the biological response elicited when an individual perceives a threat to its homeostasis. The threat is the stressor.*" Three general stages define the stress response: the recognition of a stressor, biological defense and the consequences of the stress response (Moberg, 1985).

When the central nervous system perceives a potential threat to homeostasis, the stress response begins. A biological response is developed consisting of the combination of four general biological defenses: the behavioral response, the autonomic nervous system response, the neuroendocrine response or the immune system response. The behavioral response is the first and most biologically economical response: the animal can avoid a stressor by removing itself from the threat like escaping from an enemy, seek a shade when the body temperature rises…not all the behavioral responses are adapted for all stressors, they may be limited (Moberg, 2000).

The autonomic nervous system is the second line of defense affecting a number of biological systems, including cardiovascular system, exocrine glands and adrenal medulla and resulting changes in heart rate, blood pressure and gastrointestinal activity. The pituitary hormones, that their secretion is directly or indirectly affected by the stress, regulate all these modifications (Matteri et al., 2000) implicating an altered metabolism (Elsasser et al., 2000), failed reproduction (Rivier, 1995) and immune competence (Blecha, 2000).

During a stress, a shift of biological resources away from biological activities is called "biological cost of stress". It can be negligible when the stress is of a short term, but when the stress is severe, it will represent a significant burden to the body (Moberg, 2000).

2. Heat stress in pigs

Animals, being homeotherms, can maintain a constant body temperature through thermoregulation, a balance between heat production and heat loss mechanisms (Reanudeau et al., 2012). Energy is used for maintenance and production of meat, milk, egg wool and fat tissue. As the efficiency of metabolizable energy (ME) utilization for maintenance and production is not equal to 100%, the ME not retained in the body or in a product is lost as heat (Reanudeau et al., 2012). As an animal can lose heat through evaporation, convection, conduction and radiation, the efficacy of heat loss process depends on the level of humidity in the surrounding air for evaporation and on the animal surface area, surrounding air and objects for convection, radiation and conduction (Curtis, 1983).

When the temperature rises above the upper limit of the thermoneutral zone, the animal is no longer able to regulate its body temperature. Depending on the duration and intensity of the thermal challenge, the animal responds differently: in acute heat exposure, the animal shows an increase in body temperature up to critical values (Collin et al., 2002) and short-term changes in physiological, behavioral and immunological functions (Renaudeau et al., 2012). In contrast, when exposed to a long period of high ambient temperature, increased body temperature decreasing afterward to a dynamic steady state is noticed accompanied by reduced performance and metabolic rate, changes in the cardiovascular system and behavior responses and in general morphology of the animal (Renaudeau et al., 2012).

Other factors related to animal characteristics (i.e. genotype, sex) or breeding conditions (feeding, housing conditions, management…) in addition to the animal's age (heavier pigs have a lower ability to dissipate heat having a higher subcutaneous fat tissues and higher energy intake relative to maintenance requirements) vary the pig's response to high ambient temperature (Collin et al., 2002).

Thermoregulation will result in physiological and metabolic responses having negative consequences on animal productivity and health (Renaudeau et al., 2012). For example, the respiratory ventilation rate is increased as an early response. Renaudeau et al (2012) showed an increase in the amount of air passage through the upper region of the respiratory tract via a rapid shallow breathing called thermal polypnea. With further increase in ambient temperature, a slower deeper panting phase (thermal hyperpnea) increasing the alveolar ventilation rate is adapted. Consequently, the heat loss through evaporation is improved but a respiratory alkalosis in the blood may occur leading to a moderate to severe dehydration leading consequently to a hemoconcentration (Sreedhar et al., 2013). In addition, as heat applied to the animals is enough to produce some stress, their leucocytes levels increase two folds as response (McGlone and Pond., 2002). In pigs, the normal levels of leucocytes are estimated between 10 000 and 15 000/µL.

3. <u>Heat stress and nutrition</u>

Pigs exposed to a high ambient temperature will decrease immediately their feed intake, which represents according to Baumgard and Rhoads (2013) an adaptive response across species in order to attempt to decrease the metabolic heat production. In consequence, the growth rate (Renaudeau et al., 2012), average daily gain (Le Bellego et al., 2002) and feeding time will decrease (Collin et al., 2001) as shown in figure 1.

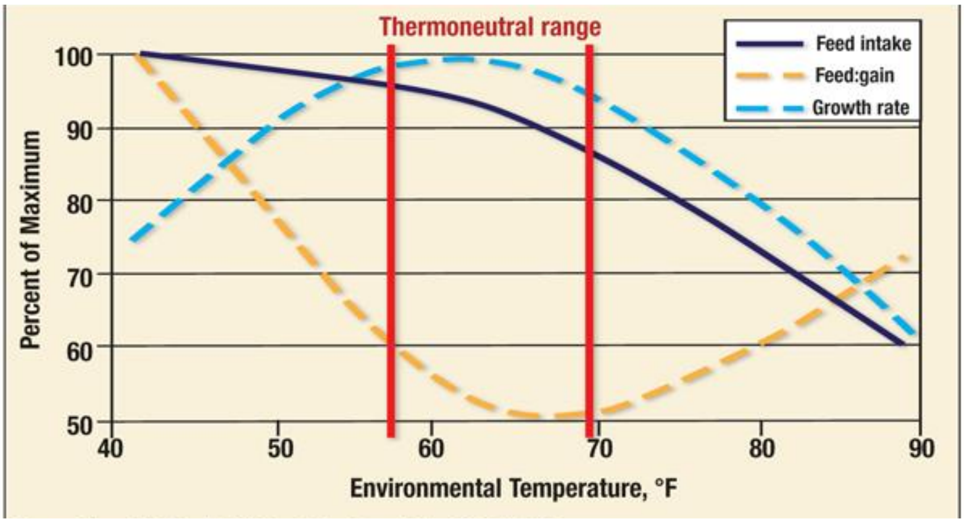

Figure 1 Effect of environmental temperature on growing-finishing pigs

Heat stress effect on carcass composition

The high ambient temperature can affect the energy distribution between protein and fat deposition, where in this condition, the protein retention decreases due to its high requirement of energy in comparison with the lipid deposition (Le Bellego et al., 2002). The metabolic utilization of crude protein or fiber leads to an increase of heat more than the utilization of starch or fat (Renaudeau et al., 2011) which will result during a heat increment an impaired synthesis of proteins, DNA and RNA (Mondovi et al., 1969) and increased plasma urea nitrogen in pigs (Pearce et al., 2013b). Consequently, the muscle mass is reduced and the adipose tissue is increased (Collin et al, 2001).

4. Heat effect on intestinal function

Decreasing the feed intake in animals can cause many modifications in intestinal functions, transport and morphology leading to an increased risk of developing bacterial sepsis (Ferraris and Carey, 2000).

The first organ affected by heat stress is the gastrointestinal tract where post-absorptive metabolism and tissue accretion can be modified by reduction of appetite, intestinal function and integrity and an elevated risk of endotoxemia (Gablar and Pearce, 2015).

The integrity of the intestinal barrier is compromised by the reduced flow of blood and nutrients to the intestinal epithelium (Pearce et al., 2013b). As the blood flow is reduced in the intestinal organs, the kidneys are not able to function properly influencing the creatinine excretion, which will produce a high level of this metabolite in the blood (Sreedhar et al., 2013). The tight junction protein complexes play a major role in normal barrier function and integrity where any modification will lead to increased pathogen amount and endotoxemia. As a result, the passage of luminal content (e.g. lipopolysaccharide (LPS) from Gram-negative bacteria) into the portal and systemic blood will increase (Hall et al., 2001). For example, during 7 days' experiment, Pearce et al (2013b) noticed an elevated blood endotoxin presented in figure 2.

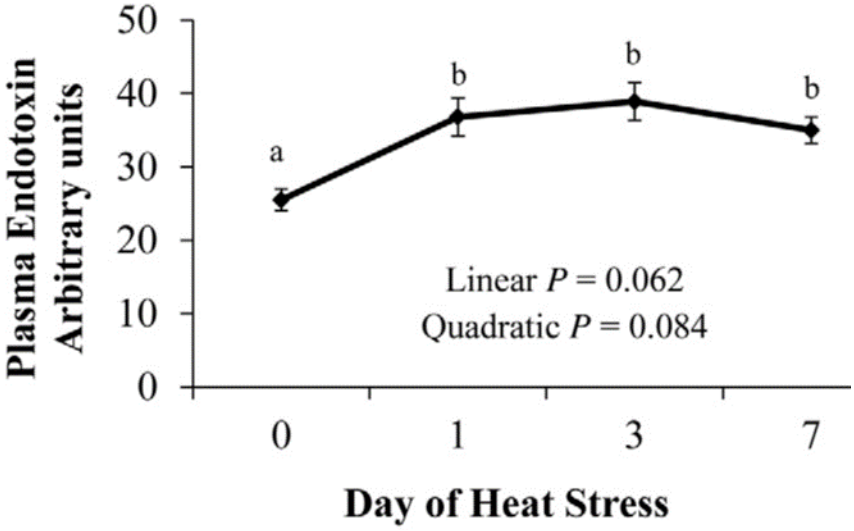

Figure 2 Elevated blood endotoxin during 7 days of heat stress (Adapted from Pearce et al., 2013b)

Another consequence of hyperthermia is hypoxia, which consists of depriving visceral organs of oxygen due to the modifications of blood and oxygen flow (Hall et al., 1999). This oxygen and nutrient restriction will result in ATP depletion, oxidative and nitrosative

stress (Hall et al., 2001), inflammation, height reduction of the intestinal villi (Pearce et al., 2013b) and damages in cellular proliferation and membrane function (Sonna et al., 2002). In conclusion, the nutrient digestion and absorption are reduced.
The changes in the intestinal integrity after the heat stress in growing pigs are resumed in Figure 3.

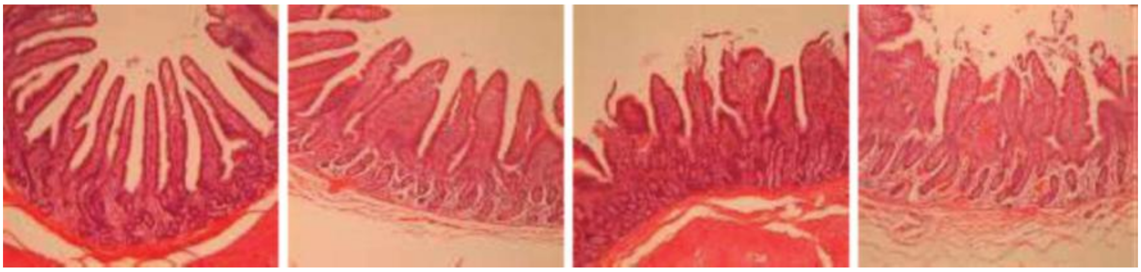

Figure 3 The changes in the intestinal integrity after heat stress in growing pigs (Adapted from Pearce et al., 2014).

Another consequence of heat stress is an elevated Na^+/K^+ ATPase activity to regulate the osmotic homeostasis membrane potential and active nutrient transport (Pearce et al., 2013b).

5. Cellular heat stress response

High ambient temperature affects cell functions. Pandey et al (2012) described that the cell´s biological function and metabolism are altered leading to an oxidative damage and apoptosis-necrosis. The balance between oxidation and anti-oxidation in cells is disturbed leading to excessive production of free radicals (Ganaie et al., 2013). In addition, Moon et al (2010) described that heat stress limits the oxidation resistance of the body by destroying the oxidative respiratory chain.

Although cell´s function and protein synthesis were disrupted such as unfolding proteins, impairing the protein synthesis and other modifications, heat stress stimulates some protein synthesis like HSFs: heat shock factors (Pirkkala et al., 2001), expression of heat shock proteins (HSPs), proteins related to acute homeostatic response (Collier et al., 2008). Under normal conditions, HSPs are present in small quantities in cells but when they are jeopardized their amount increase briefly to recover the natural form of proteins (Moseley, 1997). Their presence in high amount is an indicator of the duration and the strength of the heat stress (Mizzen and Welch 1988).

6. <u>Effect on metabolism</u>

Heat stress activates the sympathic nervous system and the release of catecholamine and glucocorticoids (Breinekova et al., 2006). Exposed to a stressor, a corticotrophin-releasing hormone is released stimulating adrenocorticotropic hormone (ACTH) from the pituitary gland thus activating the adrenal cortex to release cortisol (Becker et al., 1985). Consequently, metabolisms are altered contributing to changes in feed intake, weight gain and carcass lipid quality, as the stress activated hormones regulate processes like growth, immunity and intermediary metabolism (including gluconeogenesis, glycogen synthesis and lipogenesis).

In a given temperature, two pigs of different weight will have different feed intake and gain. Heitman and Hughes (1949) described that a pig of 45 kg of live weight continued to gain weight at 39^{0}C whereas a finishing pig did not grow at all which indicates that lighter pigs are less sensitive than heavier pigs in high ambient temperature (Ingram, 1974). As shown in figure 4, the pigs' feed intake was more affected in heavy pigs than in lighter pigs when the ambient temperature increases. It is more difficult to heavy pigs to dissipate heat due to the lower surface in comparison to the body mass when the pig is bigger (as lower the ratio surface/body mass more difficult for dissipating heat).

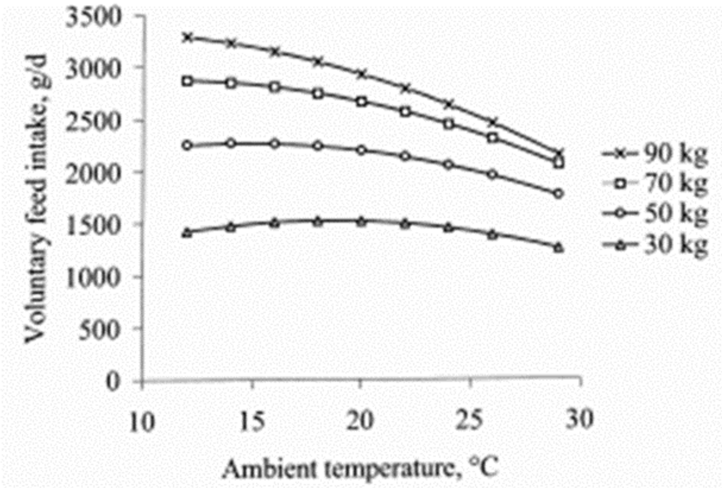

Figure 4 Relationship between body weight and ambient temperature (Adapted from Quiniou et al., 2000).

Lipid metabolism

Causing a well-described increase in stress and catabolic hormones (epinephrine, cortisol, and glucagon); HS induces many changes in carcass composition, blood lipid profiles and lypolitic capacity (Beede and Collier, 1986).

In hyperthermic conditions, Kouba et al (1999) noted a reduction in *de novo* fatty acid, Acetyl-CoA-carboxylase and stearoyl-CoA-desaturase in adipose tissue and liver. These two enzymes catalyze the first step of fatty acids synthesis and the synthesis of monounsaturated fatty acids from saturated fatty acids respectively. In consequence, changes in lipid metabolism are detected.

Generally, an animal fed inadequately will mobilize its adipose tissue and NEFA level is increased (Vernon, 1992). As the thyroid hormones stimulate lipolysis and NEFA utilization, a sharp reduction of thyroid hormones induced by heat stress (Pucci et al., 2000) will contribute to impair the mobilization of adipose tissue (Sanz Fernandez et al., 2013) and decrease the blood NEFA (Pearce et al ., 2013 a).

A reduction of ATGL gene expression, a major enzyme in the lipolytic process, indicates a decrease in circulating markers of adipose tissue mobilization during heat stress (Zimmerman et al., 2004). In addition, an increase in adipose tissue lipoprotein lipase indicates an incremented capacity to uptake and store intestinal and hepatic derived triglycerides by the adipose tissue of hyperthermic animals (Sanders et al., 2009) showing a slightly thicker back fat with a greater lipid content and an increase in VLDL-lipid concentration. Xin et al (2016) showed that plasma concentration of cholesterol, triglycerides and low-density lipoprotein cholesterol (LDL) didn´t differ in high ambient temperature contrarily to Pearce et al (2013 a) who showed that circulating triglycerides and cholesterol were increased. In addition, high-density lipoprotein (HDL) was lower and very low-density lipoprotein (VLDL) was higher (Xin et al., 2016).

The effects of a high ambient temperature will differ on pigs upon their weight. For example, Christon (1988) showed a little effect of HS on carcass tissue mass in young pigs, whereas in finishing pigs, there was an increment in adipose tissue accretion and the carcass nitrogen content was reduced.

Carbohydrate metabolism

As metabolic heat production from amino acid oxidation is high, and without any lipid mobilization, the favored fuel during hyperthermia is glucose (Streffer, 1988). Rowel et al (1986) showed, during a heat stress, an increase in glycogenolysis and/or gluconeogenesis leading to a hyperglycemia during the heat load (Marple et al., 1974). Contrarily, Pearce et al (2013 a) showed that, during high ambient temperature, the overall blood glucose decreases, it enters inside the tissues in order to provide the energy needed (Saunders et al., 2001). However, as feed intake and blood glucose are diminished, the ability of the glucose to meet the production requirements will be reduced (Saunders et al., 2001).

Due to the deleterious effect of heat stress on the intestinal health, the immune system, a potential glucose utilizer, is stimulated. As a result, the immune cells become obligate glucose utilizers, which alters the hierarchy of fuel requirements and induce a whole body shift in nutrient partitioning to spare the glucose for the immune system (Baumgard and Rhoads, 2013).

Protein metabolism

Environmental hyperthermia reduces muscle protein synthesizing machinery, RNA/DNA synthesis capacity (Streffer, 1982) and body protein metabolism and deposition (Xianyong et al., 2005). It was also noticed an increase in plasma markers of muscle breakdown in pigs (Hall et al., 1980) and blood urea nitrogen which indicate an increased skeletal muscle catabolism. The proteins and amino acids were catabolized to compensate the inadequacy of energy for maintenance (Cottrell et al., 2015).

The damage observed at the level of the intestine (explained above) will also result in increasing the epithelial cell shedding and endogenous intestinal loss of amino acids especially arginine, phenylalanine, histidine and threonine whereas the reduction in villi height will affect the protein digestion and amino acids absorption (Morales et al., 2016). The intestinal cells, mainly absorptive (enterocytes), enteroendocrine and mucoproteins secreting cells capable to complement the protein digestion and perform the amino acids absorption, are reduced during heat stress resulting in a greater endogenous intestinal loss of amino acids indicated by the reduction in expression of amino acids transporters (Morales et al., 2014).

The effects of heat stress will differ depending on its duration; Tabiri et al (2003) showed that a short-term heat stress would rise the protein degradation and reduce protein synthesis and retention, plasma level of aspartic acid, serine, tyrosine, and cysteine in finishing pigs. While Geraert et al (1996) indicated that long-term heat stress will block protein synthesis, diminish protein decomposition and amino acids level in the blood, and will increase the plasma level of aspartic acid, glutamic acid and phenylalanine.

Insulin

In order to meet the energy requirements during a heat load, catecholamine such as epinephrine are produced inhibiting insulin release (Katsuhiko et al., 1982) to increase gluconeogenesis/glycogenolysis and adipose tissue lipolysis to provide glucose for extra-hepatic tissues and fatty acids for skeletal muscle respectively (Brockman, 1986). However, as glucose is more efficient to provide ATP than fatty acids, the adipose tissue is not mobilized and the carbohydrates are more utilized (Baumgard et al., 2013).

The effect of heat stress on insulin is contradictory: for example, Pearce et al (2013a) and Sanders et al (2009) showed that the circulating insulin increases while Mersmann (1998) showed that it decreases. But both of them agreed that the insulin sensitivity in adipose tissue is reduced on the contrary of Sanz Fernandez et al (2015) that showed that insulin sensitivity is improved as glucose is needed to maintain euglycemia during heat stress.

Li et al (2006) described that a contradictory state occurs in heat stressed pigs as the insulin, an anabolic hormone, increases in heat stress, a catabolic condition. The reason of this conflicting state is not clear although it might be explained by insulin´s role in the activation of cellular stress responses.

As the immune cells become obligate glucose utilizers (mentioned above), they´ll become insulin sensitive whereas adipose tissue and muscle will become refractory to insulin and the glucose utilization in the immune system may exceed that of systemic tissue (Baumgard and Roads, 2013).

Cortisol

Circulating cortisol increases in all stressful conditions in pigs compromising animal welfare (Claus and Weiler., 1994).

During the acute phase of heat stress, serum concentration of cortisol increases inducing neoglucogenosis and higher glucose utilization. On the contrary, during the chronic phase, it decreases in order to prevent metabolic heat production (Habib et al., 1992).

Many researchers did not agree about the variation of cortisol concentrations; for example, El-Nouty et al (1980) showed that, despite the duration and magnitude of heat stress, there was a great decrease or no effect of the heat stress on corticoid levels indicating an individual inability to regulate cortisol secretion in stress conditions. Collier et al (1982) observed, during the first two hours of heat stress, a 68% increase in cortisol and after 48 hours normal values were reached until the end of the stress conditions. On the other hand, in Fagundes et al (2008) experiment, it was shown that the serum cortisol increases in both treatments (heat stress temperature and comfort temperature) showing a higher increment in serum cortisol concentrations in heat stressed pigs. At the end of the experiment, it decreases in both treatments. These results were in consistency with those of Cook et al (1998).

7. Iberian pigs

Iberian pig is of a high quality due to the high levels of fat and high concentrations of myoglobin and iron in their muscles (Ventanas et al., 2001). In addition, the high deposition of oleic acid in their tissues because of the intake of natural feeds such as grass and acorns during the last stage of fattening lead to a high quality dry cured products (Ruiz et al., 1998). For these reasons, the Iberian pig production increased 25% in the last 4 years leading to high profits (AICE, 2004). However, the production of "animales de bellota" is and will be insufficient to cover the actual and predictable market demand.

While the Spanish industry of white pig is mostly oriented to the production of lean meats, practically no exists the heavy pigs oriented to the charcuterie. For that, exists a large difference in meat products quality between the white pigs killed with lightweights and pure Iberian pigs fed with acorn. The charcuterie demands a higher quality and intermediate types that can be achieved from heavy pigs of white breeds like in France, Italy … or with the Iberian genotype with a production system based on the alimentation with feed, which has a short duration and less cost of the extensive system (in the mountain, fed acorn) but with lower quality (Lopez Bote et al., 1998).

Flores et al (1988) described that the feed of Iberian pigs is similar to the white pigs in cereals (corn, barley, wheat) and low content of fat (2-3%) thus the energy contribution is based on the carbohydrates favoring the endogenous fat synthesis. In consequence, a high percentage of saturated amino acid is produced determining that the consistency of fat of the carcass is superior to those of the mountain.

Iberian pigs can be crossed with other breeds. Hence, Iberian pigs crossed with Duroc present an increased growth, lean performance, growth rate and greater length of carcass, length and perimeter of ham and percentage of all the noble pieces. In addition, the degree of infiltration of fat is increased (Benito et al., 2004).

8. Effect of adding fat to the diet

In commercial industries, adding fat to diets of growing finishing pigs depends mainly on ingredient prices, the expected biological effects and feed processing systems (De la Llata et al., 2001) and its effect on the growth performance depends mainly on the stocking rate, fiber content of the diet and other environmental conditions (Campbell, 2005).

In general, to adapt a certain diet to high ambient temperature, the energy content and heat increment should be considered where, in this condition, a diet should be high in energy and the heat increment should be at a minimum level (Coffey et al., 1982). Having a lesser heat increment than either carbohydrate or protein, a fat-supplemented diet may be beneficial during heat stress where it will increase the availability of the nutrient for tissue synthesis in animals (Coffey et al., 1982). In addition, Holmes and Grace (1975) concluded in their experiment that the supplementation of fat in diets has a low heat increment and a stimulatory effect on feed consumptions on animal maintained in warm environments. When increasing the dietary DE using fat rather than carbohydrates, there was an evidence of an increased feed efficiency. In addition, as described by Coffey (1999), pigs consume feed in order to meet their energy requirements and as fat is high-energy ingredient, when increasing it in the diet, the animal´s feed intake will decrease consuming the same amount of energy required. According to Campbell (2005), the diets with added fat led to a decreased feed intake, an increased growth rate and the feed efficiency was improved by 2-6% of finishing pigs but there was no effect on the back fat thickness. Baudon et al (2003) obtained the same results during the growing finishing phase, where the fat inclusion resulted in greater efficiency of gain and greater carcass yield (dressing percentage). However, De la Llata et al (2001) showed distinctive results than the previous depending on the growth phase. They showed that there was no effect of adding fat during the two phases on pigs´ feed intake, and although the ADG increased during the growing phase, the supplemented fat did not affect it during the finishing phase. Moreover, the dietary treatment containing 6% of added fat led to an increased back fat depth, a decreased lean percentage but no significant difference was seen in hot carcass yield. Furthermore, Brooke et al (2015), working in a normal ambient temperature with Landrace x Large White pigs, found that ADG increased while there was no effect on feed intake and feed conversion ratio and dressing percentage during the growing finishing pigs. Contrarily to all previous studies, Myer and Combs (1991) found that there was no effect on pigs´ ADG with added fat diet. Moreover, when using a higher fat supplementation of 10%, Overland et al (1999) didn´t find any effect on feed intake although the FCR and ADG were improved and the live weight increased.

Pigs fed ad-libitum with supplementary fat addition represented an increase in carcass composition, back fat thickness and whole body fat content (Hanke et al., 1975). This is largely attributed to the improved efficiency of synthesizing adipose tissue from dietary fat compared with carbohydrates (Hillcoat and Annison, 1973) and the increased energy intake frequently observed in fat supplementation (Hanke et al., 1975). In addition, they noted a 9, 5 and 8 % improvement in daily gains, ME intake and efficiency of energy utilization after adding a 5% of fat to the diet of heat stressed pigs. However, adding fat to the diet lead to a high cholesterol and triglyceride levels in the blood (Eisinger et al., 2014).

In addition to the growth parameters affected by the supplemented fat, the fatty acid composition of subcutaneous fat is also affected. As described by Sardi et al (2007), when using a high oleic diet, higher oleic and MUFA levels and a decreased SFA were obtained. However, Realini et al (2010) showed that PUFA, linoleic and linolenic acid, n3, n6 and n3/n6 ratio were increased when fat was added to the diet.

The fatty acid composition of the diet is reflected in the fatty acid profile of the subcutaneous fat (Sardi et al., 2007). The fatty acid profile, particularly the oleic acid, of tissue lipids from Iberian pigs affects technological, sensory and nutritional quality of meat since the physical state of fat (liquid or solid) highly depends on it. For the Iberian pig fat, the oleic acid is the main contributor to fat fluidity (Ventanas et al., 2005) giving a soft and oily aspect to the fat. These features (soft and oily fat) are accepted and highly valued by the consumers of Iberian dry cured ham due to the high oleic content and the desirable flavor (Ruiz et al., 2002).

9. Effect of restricted feeding

The effect of feed restriction on production indices depends mainly on its degree, duration (Campbell et al., 1983) and the growth potential of the pigs in question (Donker et al., 1986). For example, when Daza et al (2003) applied a restriction of 25%, the feed intake and the average daily gain were reduced while there was no effect on feed conversion ratio, which it was also obtained by Cho et al (2006) using animals from 50 kg to slaughter weight. However, with a more severe restriction of 46%, Daza et al (2003) observed that the feed conversion ratio increased. Serrano et al (2009), applying a 18% restriction on Iberian x Duroc pigs showed no significant difference on the feed conversion ratio while the FI and ADG were decreased. Moreover, Ugwu and Onuimonyi (2009) showing the same results concerning the feed intake and the feed conversion ratio, observed a slight decrease in the feed conversion ratio.

Furthermore, the carcass traits and the fatty acid composition are affected by the feed restriction. Serrano et al (2009) showed that although the dressing percentage and the fat thickness were not affected, loin thickness increased in restricted pigs also showed in Daza et al (2006) experiment applying a 50% restriction on Iberian pigs of initial body weight of 55 kg.

10. Behavior modification in heat stressed pigs

Dirtiness

In high ambient temperature, pigs having a limited capacity to sweet adapt a wallowing behavior in the dunging area in order to cover their body with a fresh layer in order to increase the evaporative heat loss, which consequently lead to a high dirtiness (Huynh et al., 2005). Huynh et al (2005) focused on the percentage of area of solid floor fouled with excrement where he noticed that the floor is dirtier of 0.7% with increased temperature of one degree Celsius. At the same time, pigs reduced urination, it was explained that the reason for this decrease is the dissipation of water containing body heat by the increased respiratory frequency (Huynh et al., 2004). In addition, the type of flooring is essential, where the animals prefer to lay on floor without bedding rather than bedded floor (Debrecéni et al., 2014).

Furthermore, it was described that pigs, adopting an unstable position when excreting (Baxter, 1982), seek areas far than the commotion area, that is most commonly near to the feeding area, to excrete and they lie away from wet areas like where the waters are sited. For this reason, pigs tend to urinate, defecate and drink in the same area of the pen (Fritschen, 1975). Additionally, Hacker et al (1994) reported that, in pens with open partition, where the pigs can see and directly communicate with neighboring pigs, they tend to excrete along with these opening partitions due to the marking behavior, thus indicating to the neighbors their territorial limits.

Lying behavior

In normal conditions, pigs are mostly sleeping and resting during 19 hours:

- 55 - 60% of the total time of young pigs is spent lying (Blackshaw, 1981)
- 65% of total time of pigs aged 22 weeks is spent lying, 7% sitting (Morrison et al., 2007)

As temperature increases, the animals will spend more time lying using more space, avoiding contact with pen mates and reduce their activity levels (walking, drinking and fighting behavior) (Spoolder et al., 2012). At the same time, Huynh et al (2005) related the reduced activity during high ambient temperature conditions to the increased lying behavior.

Huynh et al (2005) reported that lying on the lateral side is a strategy adapted by the pigs to dissipate the increased body heat brought about by the increased ambient temperature by increasing the body contact with a cooler surface, the floor. In addition, the lying posture differs depending on the temperature: when the temperature increases one degree Celsius, the lying posture of the animal will be distributed as 72% lying laterally, 15.4 laying semi-laterally and 12.4% lying sternly. These results were also consistent with the study of Close et al (1981). Moreover, with each increment in ambient temperature of one degree Celsius, the animal will decrease the huddling behavior and increase wallowing.

A number of factors including the pen design, location of the feeder and drinker, and environmental conditions determine the lying zone of the pig (Costa et al., 2004). The pigs tend to lie in dry areas, far than the commotion areas that are mostly near to the feeders.

Interactions

The reduced activity levels were described by Pederson et al (2003), where it was observed that animals reduce their activity from 20% to 8% when temperature rises to 28^{0}C showing a 12% increase in lying behavior. In addition, Ingram et al (1980) noticed that during the dark period the animal would have higher activity level as the temperature decreases.

In addition, increased weight will induce a higher number of lying pigs (Ekkel et al., 2003) also increasing age will reduce aggression, ear manipulation and other social interactions (Lyons et al., 1995).

At youngest age, the animals are more aggressive having the highest frequencies of occurrence of social behavior-aggression and fighting and thereafter, these behaviors are decreased as showed by Debrecéni 5 days' experiment (2014) in figure 5.

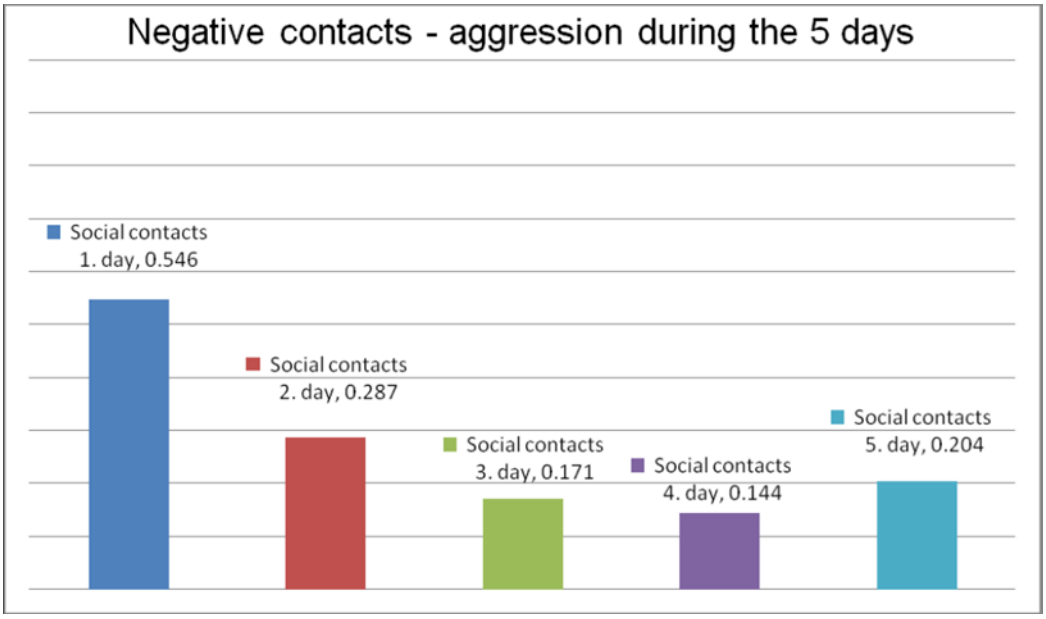

Figure 5 Decreased social aggression behavior during 5 days of heat stress (adapted from Debrecéni et al., 2014)

Feeding behavior

During a heat stress, pigs eat mostly during the morning, later in the day and in the hours of darkness (Fraser, 1984) and decrease the consumption time (duration of the meal) as well as the occupation time of the feeding station (Guiniou et al., 2000). Moreover, pigs drink mainly at mealtime (Fraser, 1984).

Figure 6 shows the daily activities of fattening pigs influenced by heat: animals spend the most of their time lying than eating than standing. The least time was spent on sitting and moving.

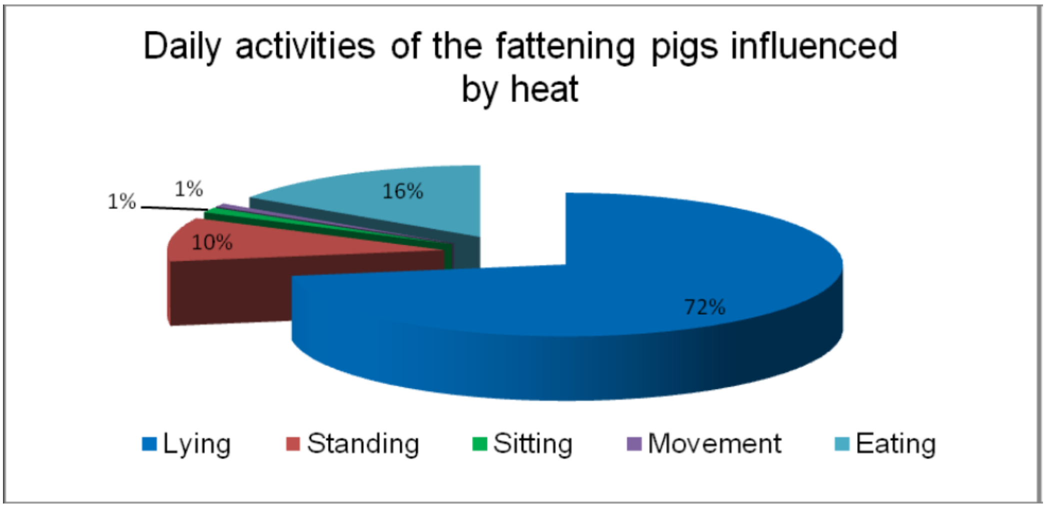

Figure 6 The daily activities of fattening pigs influenced by heat in percentage (adapted from Debrecéni et al., 2014)

II. Materials and methods

The experiment was conducted at the "Institut de Recerca i Tecnologia Agroalimentàries" (IRTA) located in the city of Monells, in the province of Girona - Cataluña, Spain. The IRTA ethical committee approved all the procedures carried during this experiment.

Animals

Seventy-two castrated males crossed (50% Iberian – 50% Duroc) Iberian pigs (of 51.1 ± 0.75 Kg of average live weight at the beginning of the study) were distributed in three rooms at the rate of four pens per room and six animals per pen. In order to identify them, in each pen the animals carry ear tags of different colors: red, orange, yellow, green, blue or violet.

Treatments

Two treatments were applied based in two different diet composition: control diet (control) and alternative diet (high fat). The alternative diet was based on decreasing starch by 5 points and increasing fat (sunflower oil high in oleic) by 5 points as well during the growing (Table 1 & 2) and finishing phase (table 3 & 4).

Table 1 Diets used during the growing phase

Ingredients	Control	Alternative
Barley	33,57	33,57
Wheat	13,41	13,41
Cornstarch	5,00	0,00
High oleic sunflower oil	0,98	5,98
Triticale	20,00	20,00
Integral sunflower	8,00	8,00
Bran	5,00	5,00
Sepiolite	1,97	1,97
Soy 47	8,79	8,79
Molasses beet	0,48	0,48
Carbonate Ca	1,74	1,74
Bioyls 70	0,15	0,15
Salt	0,33	0,33
NP - 316 - Fi	0,30	0,30
Monocalcium Phosphate	0,24	0,24
Redox - N	0,02	0,02
Liquid choline 75%	0,02	0,02

Table 2 Nutrient composition of the diets used during the growing phase

Nutrients	control	alternative
Dry material, %	90,30	90,96
Crude protein, %	14,50	14,47
Crude fat, %	2,61	7,52
Crude fiber, %	5,60	5,59
Ash %	7,00	7,05
Starch %	43,90	38,90
Carbohydrates, %	2,96	2,96
Ca%	0,98	0,98
P_%	0,44	0,44
Cl_%	0,30	0,30
Na%	0,16	0,16
LYS%	0,72	0,72
MET%	0,24	0,24
MET+CYS%	0,54	0,54
THR%	0,51	0,51
TRP%	0,19	0,19
OL_C18_1%	1,00	4,83
LI_C18_2%	0,92	1,35
R_INSAT_%	2,04	6,52
R_SATUR_%	0,37	0,76
UNS/SAT_	5,47	8,55
NE_SW Kcal/Kg	2250,00	2524,00
ME_SW Kcal/ Kg	3066,00	3340,00
VIT_A_AD IU/ Kg	2000,00	2000,00
VIT_D_AD IU/ Kg	800,00	800,00
VIT_E_AD mg/Kg	100,00	100,00

Table 3 Diets used during the finishing phase

Ingredients	control	alternative
Barley	45,00	45,00
National corn	4,59	4,59
National wheat	25,00	25,00
Cornstarch	5,00	0,00
High oleic sunflower oil	0,91	6,00
Integral sunflower	8,00	8,00
Bran	5,00	5,00
Sepiolite	1,97	1,87
Soy 47	1,85	1,84

Molasses cane	0,48	0,48
Ca carbonate	1,08	1,10
Bioyls 70	0,25	0,25
Salt	0,40	0,40
NP - 316 - Fi	0,30	0,30
Monocalcium phosphate	0,11	0,11
L-threonine	0,03	0,03
Redox - N	0,02	0,02
Liquid choline 75%	0,02	0,02

Table 4 Nutrient composition of the diets used during the finishing phase

Nutrients	**control**	**alternative**
Dry material, %	90,46	91,17
Crude protein, %	11,00	10,97
Crude fat, %	2,54	7,55
Crude fiber, %	5,98	5,97
Ash %	6,05	5,97
Starch %	48,31	44,00
Carbohydrates, %	2,41	2,41
Ca%	0,69	0,70
P_%	0,37	0,37
Cl_%	0,35	0,35
Na%	0,18	0,18
LYS%	0,55	0,55
MET%	0,18	0,18
MET+CYS%	0,42	0,42
THR%	0,40	0,40
TRP%	0,14	0,14
OL_C18_1%	1,00	5,09
LI_C18_2%	0,92	1,14
T_INSAT_%	2,00	6,36
T_SATUR_%	0,39	0,82
INS/SAT_	5,07	7,79
NE_SW Kcal/Kg	2293,00	2575,00
ME_SW Kcal/ Kg	3066,00	3348,00
VIT_A_AD IU/ Kg	2000,00	2000,00
VIT_D_AD IU/ Kg	800,00	800,00
VIT_E_AD mg/Kg	100,00	100,00

In addition, two forms of feeding were used. Half of the animals were fed ad-libitum and the other half restricted. In each room there was one pen with one of the following four combinations: control ad-libitum (CA), control restricted (CR), high fat ad-libitum (HA) and high fat restricted (HR) as showed in figure 7. The restricted group was fed a 90% of the daily feed intake of the ad-libitum group inside the same treatment (control or high fat diet).

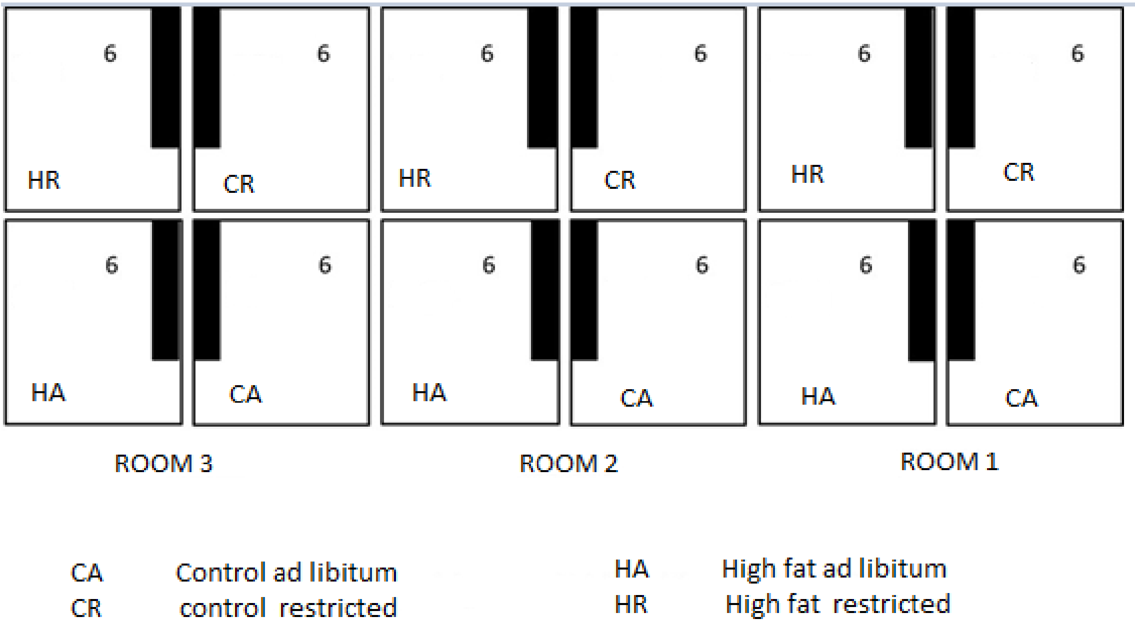

Figure 7 Scheme of the experimental design, considering the diets (control and high fat) and the regimens (ad-libitum and restricted).

Temperature

The animals were subjected for 5 hours daily at high temperatures: 30-32°C (between 10:00 and 15:00) and during the other hours the temperature was maintained at a minimum of 25°C (figure 8). Temperature was measured daily using a thermo hygrometer (HygroLog, Rotronic Hygromer TM C94, sensors Pt100 RTD (1/3 DIN), Switzerland).

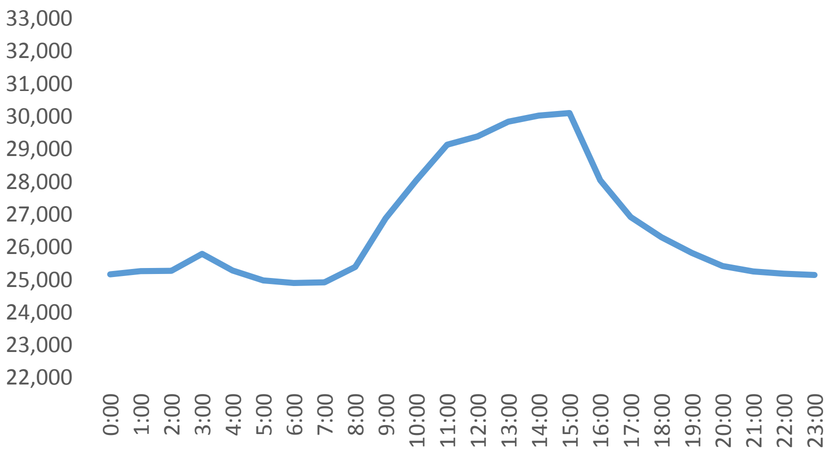

Figure 8 Temperature cycle applied during the experiment

Measurements

On arrival, the animals were weighed and distributed in the three rooms according to their weights: In room 1 the heaviest (58.2 ± 0.81 Kg), in room 3 the lighter pigs (44.3 ± 0.41 Kg) and in room 2 the animals in between (50.75 ± 0.37 Kg) and homogenized by weights within the different pens of each room, so no significant differences ($P > 0.05$) were found between treatments by weight on day 0 of the study. After a period of adaptation to the new facilities of 10 days with all pigs being fed with the control diet, the hot stress (high temperature) conditions were applied and, the treatment with the alternative diet was applied as explained above. At this moment, the animals were weighted again and no significant differences ($P > 0.05$) were found between treatments (61.6 ± 0.85 Kg). Since then, the animals were weighed once every three weeks until the end of the study at 130.2 ± 1.48 Kg.

Feed intake was measured in a daily basis by group by weighing the quantity of food given to the animals fed ad-libitum.

Just after the 10 days of adaptation and before starting the treatment of the alternative diet, a blood sample was taken for hemogram and serum for biochemical analysis (creatinine, urea, ALT, AST, total cholesterol, Triglycerides, HDL and LDL) to obtain basal values (A). Other two extra samples of serum were taken in the middle (B) and at the end of the study (C), while hemogram was studied only once more at the end of the study.

Behavioral measures

The dirtiness, based on the classification used in Welfare Quality (Welfare Quality, 2009) was evaluated every day (Annex 3):

0: animal with feces in less than 20% of the body

1: animal with feces in more than 20% but less than 50% of the body

2: animal with feces in more than 50% of the body

In addition, once a month, a complementary evaluation of welfare was made. This evaluation includes other parameters of welfare quality protocols, such as bursitis, body injuries, lameness, cough, sneezing, breathing difficulties, atrophic rhinitis, hernias, rectal prolapse, poor body condition, poor skin condition, tail biting and diarrhea.

The animals fed restricted received the feed twice a day: at 9:00 and 16:00 in the order of rooms: 3, 2 and 1. While providing the feed, the position of each animal in the row of feeders was noted (figure 9), as there was one position for each animal (6 in total). In addition, the following times were noted (see annex1):

- The total amount of time in which a pig was continuously eating without a pause or until it was displaced
- The total amount of time in which pigs finished the food provided per meal.

Figure 9 The position of pigs fed restricted in feeders numbered from 1 to 6

Figure 10 Behavioral observations

Since 50% of the animals were under an ad-libitum regime, it was necessary to combine focal with scan sampling to assess the competition for food in these animals. In this case the observations consisted in assessing the behavior of the animals from 8:00 to 18:00 in periods of two hours that were assessed at different days to avoid the fatigue of the observers (in total animals were assessed 20 hours with this system). The two hours of observation consisted of 24 scans (one each 5 minutes) and 23 focal sampling of four minutes each. Scans were used to assess which animals were using the feeder or doing other activities in all the ad-libitum pens each time and focal sampling to assess specifically in one pen each time all the activity in the feeding area, such as presence of positive or negative social interaction and time in the feeding area.

Finally, the general activity and position of the animals was as well assessed by means of a scan sampling three times per day. For this purpose, the pen was divided in 8 areas as seen in figure 11, and the behaviors considered were eating, sleeping (sternly or laterally), drinking, positive and negative interactions and others. This assessment was done always twice per day in the ad-libitum fed pigs (at 9:00 and 16:00 just after feeding the restricted ones) and in all the pigs once per day at a random hour (except 9:00 and 16:00) from 8:00 to 18:00h.

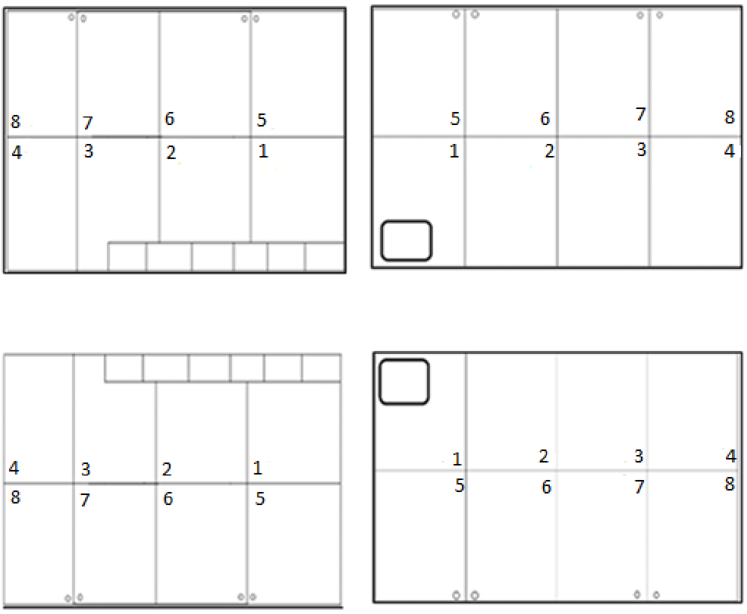

Figure 11 Scheme of pens decomposition and enumeration, considering the position of the feeders and drinkers

Meat quality

At an average live weight of 130.2 ± 1.48 Kg, which occurred after 14 weeks, the animals were killed in a commercial slaughterhouse.

Measures 24h post mortem

Meat quality was assessed on the left half of carcass of each animal slaughtered using these measures:

- pH_u
- Electrical conductivity (EC_u)
- meat color

At the level of the last rib of the *longissimus thoracis* (LT), pH_u (or ultimate pH) and electrical conductivity (EC_u) were measured using a portable pH meter (Crison, Hach Lange Spain S.L.U., Spain) equipped with a Xerolyt penetration probe and a conductimeter (PQM Future, Classpro GmbH, Germany) respectively.

In order to obtain the lightness (L*) of meat as defined by the CIE (1976), meat color was determined using a colorimeter CR-200 (Konica Minolta Inc., Japan) on loin samples that were vacuum packed and frozen for later processing.

From the left ham of each carcass, subcutaneous fat samples over the *Gluteus medius* were taken from the left side of each carcass at 24 hours post-mortem.

Following the chloroform-methanol procedure of Folch et al (1957), tissue lipids were extracted. Lipids were transmethylated with BF_3 and methanolic KOH (Morrison and Smith, 1964) and fatty acids were determined by gas chromatography (VARIANT CP3800) using a pre-column SGE (2m x 250 µm VSD Tubing) and a capillary column BPx70 (0.25mm x 0.25 µm x 30 m) and a flame ionization detector (FID). A temperature gradient with initial temperature of 80 ºC (1 min) followed by an increase at 3 ºC/min until 210 ºC and remained at this temperature during 12 min. The injector temperature was set at 270 ºC and the detector temperature at 280 ºC. The injection was in the Split mode with a ratio of 1:40, and the carrier gas was helium with a flux of 1 ml/min.
The Standard adopted was FAME Mix C4-C24 (Sigma Aldrich, St Louis, USA) and 1,2,3-Tripentadecanoylglycerol (Sigma, St Louis, USA) was used as internal standard.

III. Statistical Analysis

Statistical analyses were performed by means of the Statistical Analysis System (SAS) (SAS 9.1; software SAS Institute Inc., Cary, NC). Feed intake, Average daily gain feed conversion ratio, hematocrit, leucocytes, neutrophil/lymphocyte ratio, creatinine, urea, ALT, AST, Cholesterol, triglyceride, LDL, HDL, live weight, carcass weight, fat thickness, dressing percentage, percentage of lean, loin thickness, color (a*, b*, L*), PH, Conductivity, intramuscular fat and fatty acid composition variables were analyzed using the PROC MIXED procedure. In all cases, the models accounted for the effects of diet (alternative or control), regimen (ad-libitum and restricted) and the diet*regimen interaction. When needed, the moment (basal, at the middle of the experiment, at the end) was also considered in the models. The variables Time in finishing the meals and time until the first interruption when feeding were also analyzed with Proc Mixed, but in this case the models accounted for the effects of hierarchy, feeder number, number of changes of position, displaced and diet (control vs alternative). In the case pen dirtiness, animal dirtiness, drinking behavior, positive and negative interactions and exploratory a Proc Genmod with a binomial distribution were used, being the diet (alternative vs control), regimens (ad-libitum vs restricted), zone of the pen (1 to 8) and interactions the effects studied. In all cases, the residual maximum likelihood was used as a method of estimation and the least square means of fixed effects (LSMEANS) was used to carry out multiple comparisons. The p value for significance was fixed at $P < 0.05$.

IV. Results

The evolution of feed intake during the experiment is seen in the figure 12. It was found an effect of the diet and regimen ($P < 0.0001$) during the growing and finishing phase: Animals fed with the control diet had higher FI than those fed with the alternative one (figure 13) and animals fed ad-libitum had a higher FI than animals fed restricted (figure 14).

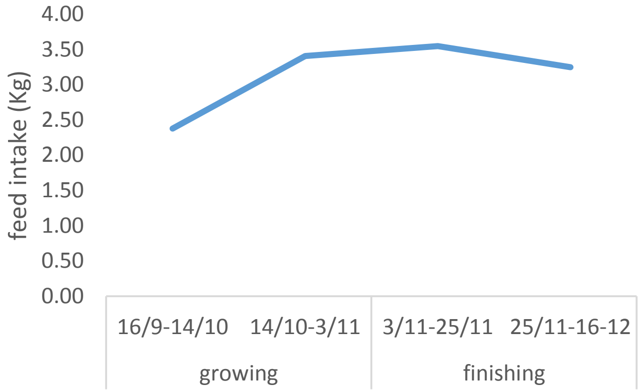

Figure 12 Evolution of feed intake during the study

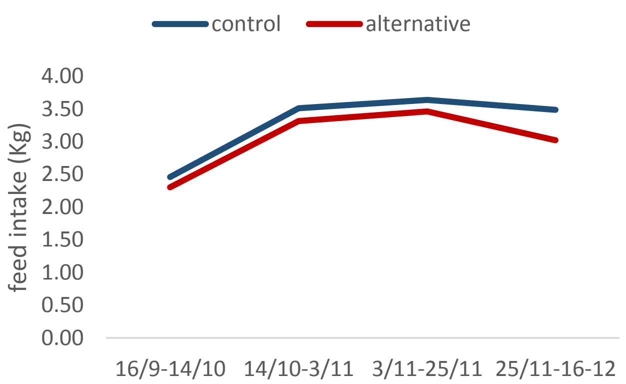

Figure 13 Feed intake of pigs fed with the alternative and the control diet

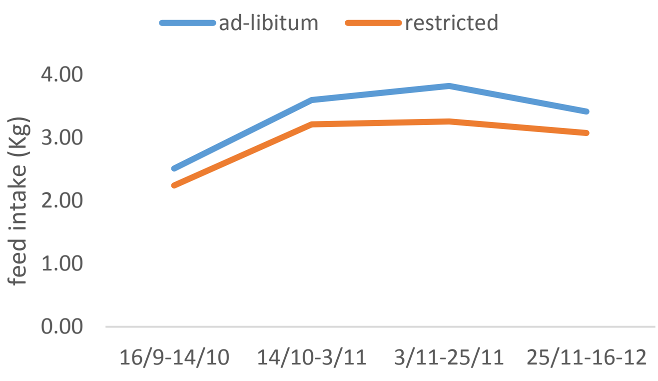

Figure 14 Feed intake of pigs fed ad-libitum and restricted

In the figure 15, it can be seen the evolution of average daily gain during the study. In comparison to the first weeks, it can be observed a reduction of 8.24% at the end of the growing period and a further decrease of 39.74% at the end of the finishing period.

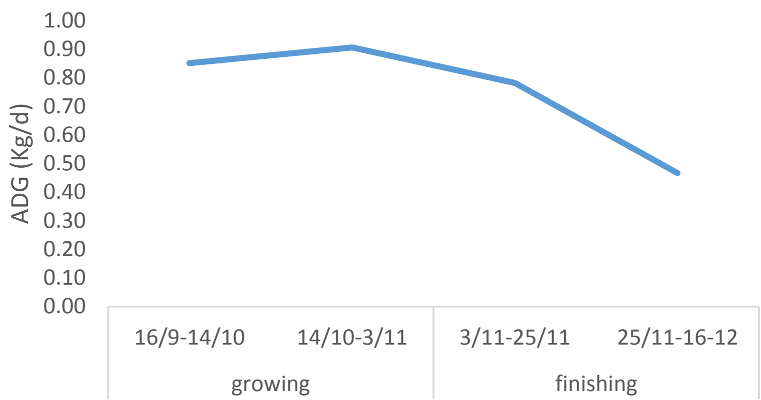

Figure 15 Evolution of average daily gain during the study

ADG was affected by regimen ($P < 0.0001$) but not by the diet, animals fed ad-libitum had higher average daily gain than animals fed restricted (figure 16).

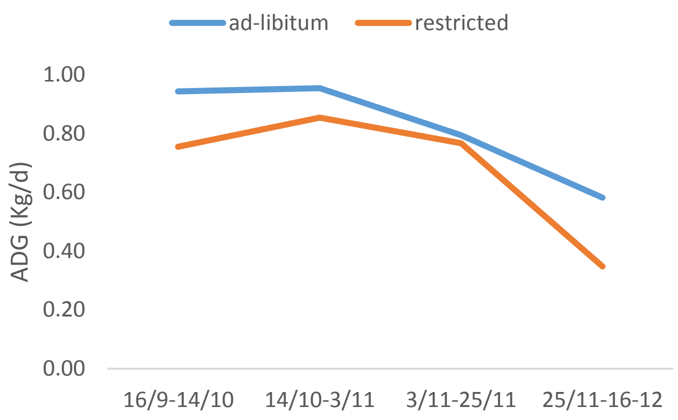

Figure 16 Average daily gain of pigs fed ad-libitum and restricted

In the figure 17 is shown the relationship between FI and ADG during the study. In both cases, it can be seen a change in the curve after the change of the diet from grower to finisher.

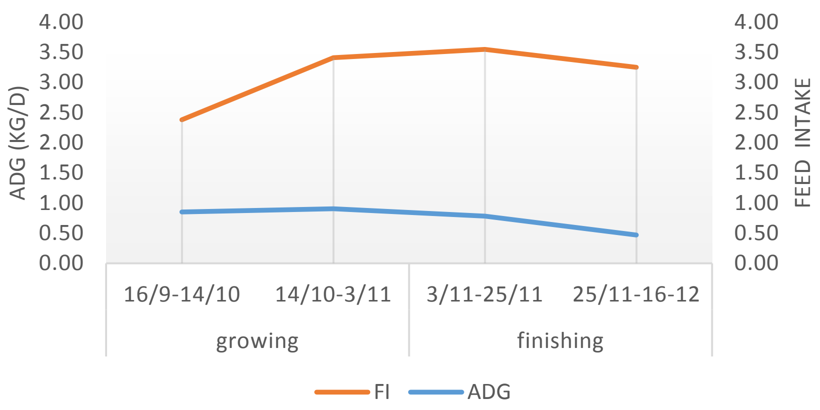

Figure 17 The evolution of feed intake and average daily gain during the study

For the feed conversion ratio, it was found an effect of the diet (P = 0.0030) in the growing period and an effect of the regimen in the finishing period (P = 0.0018). In the case of the growing period, animals fed with the alternative diet had lower FCR than those fed with the control diet (figure 18). As for the finishing period, animals fed ad-libitum had a lower FCR than the animals fed restricted (fig 19).

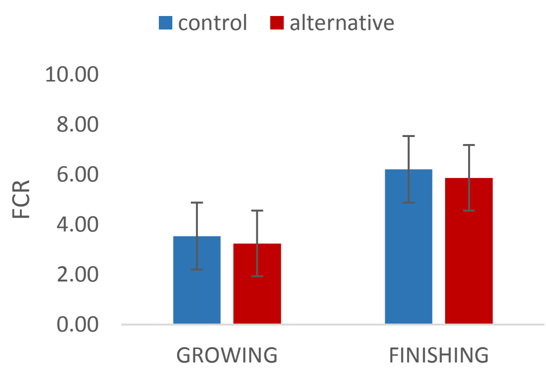

Figure 18 Feed conversion ratio of pigs pigs fed with the control diet and pigs fed with the alternative one during the growing an =d finishing phase

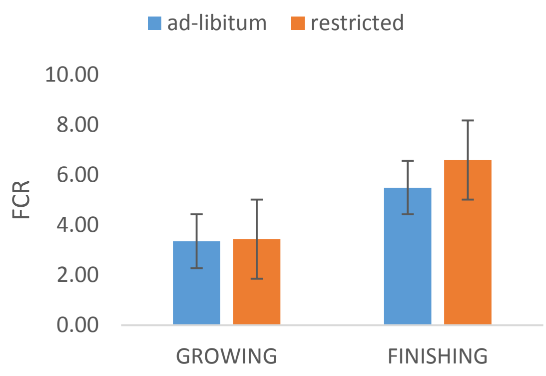

Figure 19 Feed conversion ratio of pigs fed ad-libitum and those fed restricted during the growing and finishing phase

When the two phases (growing and finishing) were not considered separately, only an effect of the regimen was found ($P = 0.0411$) showing that pigs fed ad-libitum had a lower feed conversion ratio than those fed restricted.

Hemogram

Hematocrit level increased ($P < 0.0001$) when the basal value was compared with the final one (figure 20). However, there was no effect of the diet or regimen at the beginning neither at the end of the study. In addition, Leucocytes level decreased ($P < 0.0001$) when the basal value was compared with the final one (figure 21). No differences were found when comparing between treatments.

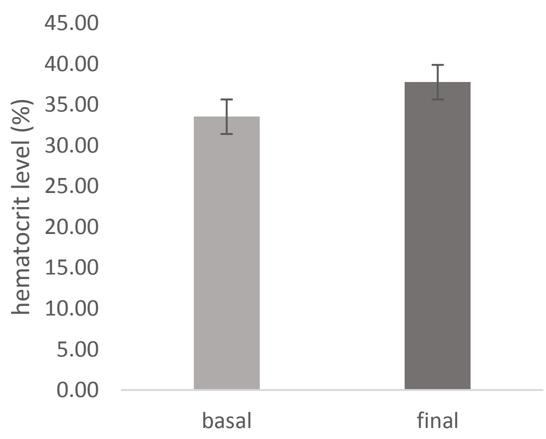

Figure 20 Hematocrit levels in basal and final sampling

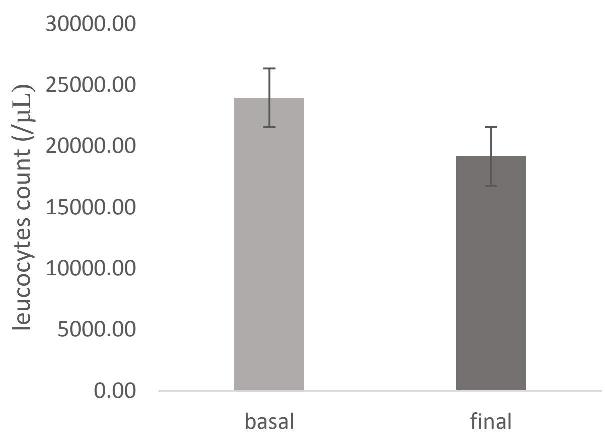

Figure 21 Leucocytes count in basal and final sampling

The neutrophil/lymphocyte ratio decreased being higher at the beginning than at the end of the study ($P < 0.0001$) (figure 22). Although there was no difference between diets, it was noticed that the neutrophil/lymphocyte ratio differed between the two regimens ($P = 0.0302$) at the final value, ad-libitum fed animals showed higher neutrophil/lymphocyte ratio than animals fed restricted (figure 23).

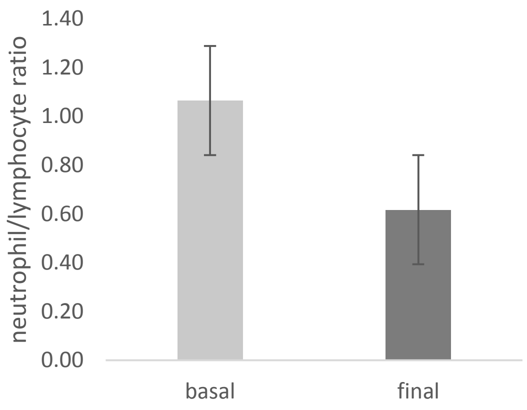

Figure 22 Neutrophil/lymphocyte ratio in basal and final sampling

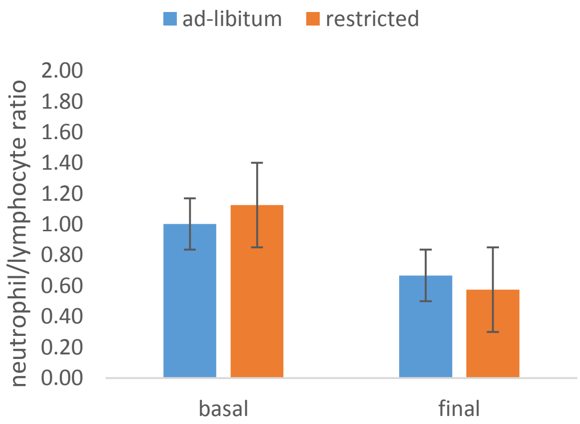

Figure 23 Neutrophil/lymphocyte ratio in pigs fed ad-libitum and those fed restricted during the growing and finishing phase

<u>Biochemical results</u>

Samples of serum for biochemical analysis were taken at the beginning of the experiment (A), at the middle before changing the diet from grower to finisher (B) and at the end of the study (C).

Creatinine level increased ($P < 0.0001$) when C is compared with A (figure 24). It increased in B ($P < 0.0001$) and a further increase was seen in C ($P = 0.00069$). No differences were found when comparing between treatments.

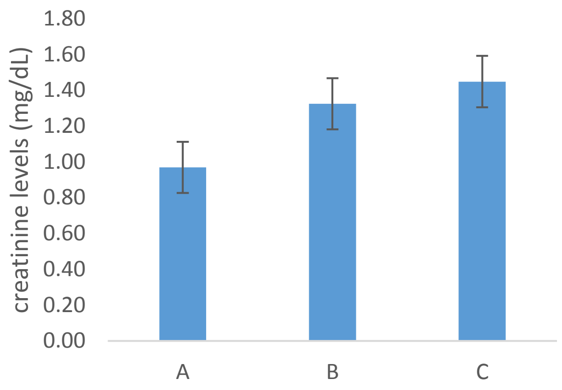

Figure 24 Creatinine levels during the three samples: at the beginning (A), at the middle (B) and at the finishing (C) of the study

During the study, urea presented higher values in B (P < 0.0001), (figure 25). It was observed that animals fed with the control diet had higher urea level than those fed with the alternative diet in C (P = 0.03), (figure 26). In addition, animals fed ad-libitum presented higher levels of urea than those fed restricted in B (P < 0.0001) and C (P = 0.0006), (figure 27).

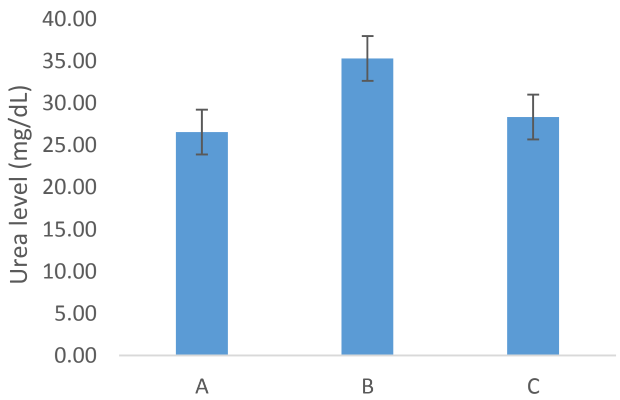

Figure 25 Urea levels during the three samples: at the beginning (A), at the middle (B) and at the finishing (C)

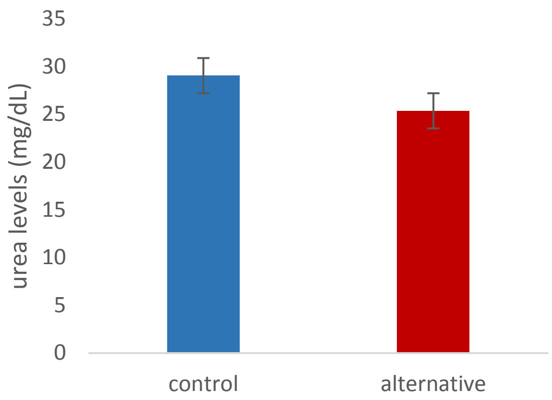

Figure 26 Urea levels during the final sampling (C) in pigs fed with the control diet and those fed with the alternative one

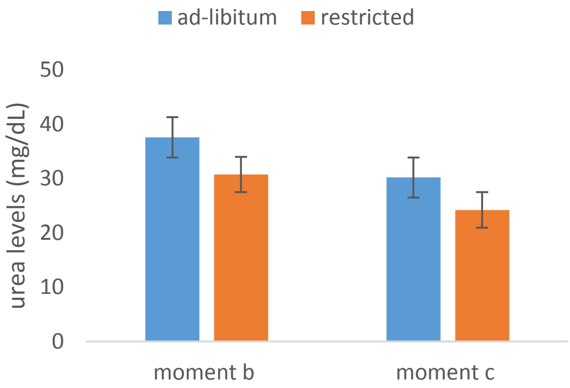

Figure 27 Urea levels in pigs fed ad-libitum and those fed restricted in the middle (B) and final sampling (C)

ALT levels increased when C was compared with A ($P < 0.0001$) but no differences were found between B and C (figure 28). In C, it was found an effect of diet ($P = 0.0119$) being higher with the control than the alternative diet (figure 29).

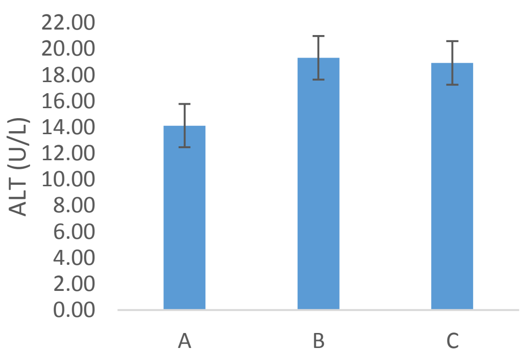

Figure 28 ALT levels during the three samples: at the beginning (A), at the middle (B) and at the finish (C).

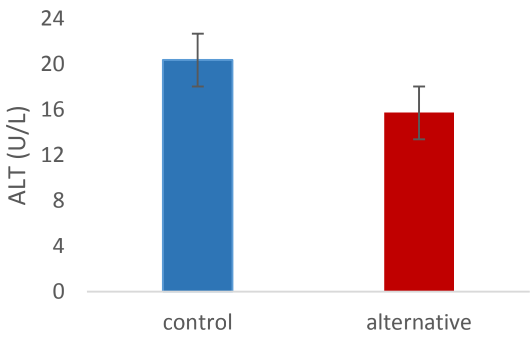

Figure 29 ALT levels in pigs fed with the control diet and in pigs fed with alternative diet at the final sampling (C).

For AST, there was no significant difference when comparing between A and C, but an increase was seen in B (P = 0.0029) and then a decrease in C (P = 0.0492) as shown in figure 30. However, no effect of treatments was found.

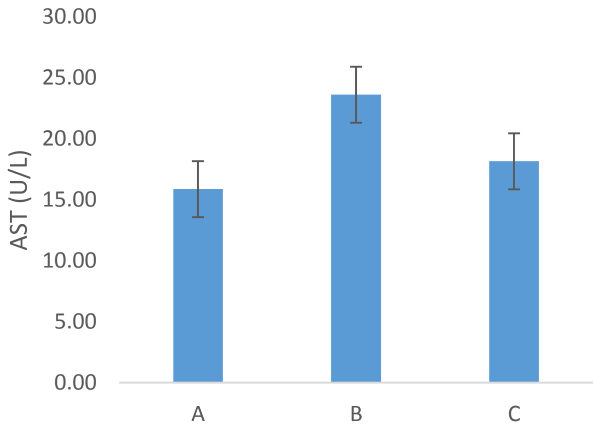

Figure 30 AST levels during the three sampling: at the beginning (A), at the middle (B) and at the finish (C)

Cholesterol level increased (P < 0.0001) during this study when C was compared with A. Although no differences were seen between A and B, cholesterol levels increased in C (P < 0.0001), (figure 31). In C, it was found an effect of the diet (P = 0.0094), animals fed with the alternative diet had higher levels of cholesterol than those fed with the control diet, and of regimens (P = 0.0263), animals fed ad-libitum had higher levels of cholesterol than animals fed restricted (figure 32).

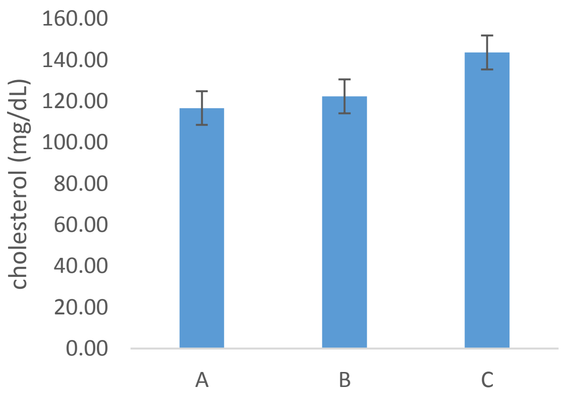

Figure 31 Cholesterol levels during the three samplings: at the beginning (A), at the middle (B) and at the finish (C).

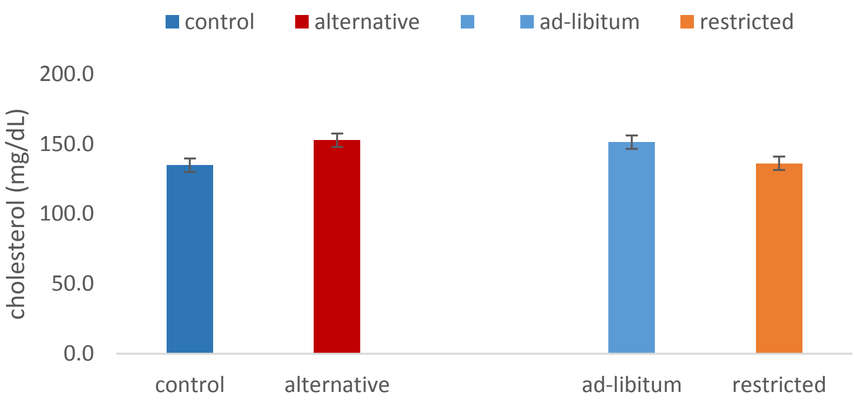

Figure 32 Cholesterol levels in pigs fed ad-libitum and in those fed restricted and in pigs fed with the control diet and in those fed with the alternative diet in the final sampling (C).

There was no difference in triglyceride level between A, B and C (figure 33). it was found effects of diet and regimen in B and C. Animals fed ad-libitum presented higher levels than animals fed restricted in B ($P < 0.0001$) and C ($P < 0.0001$), (figure 34). In addition, higher triglyceride levels were obtained when using the alternative diet than when using the control diet in B ($P = 0.0039$) and C ($P = 0.0359$), (figure 35).

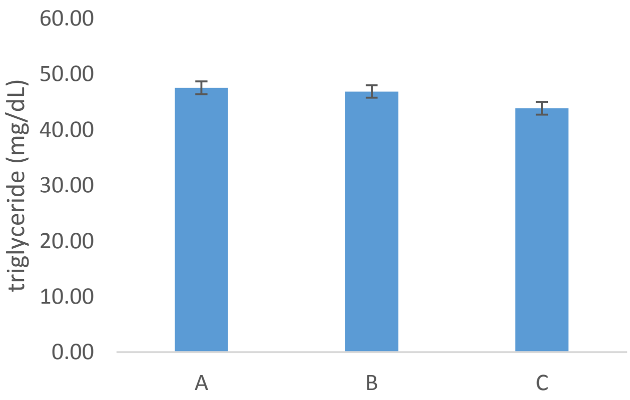

Figure 33 Triglyceride levels during the three sampling: at the beginning (A), at the middle (B) and at the finish (C).

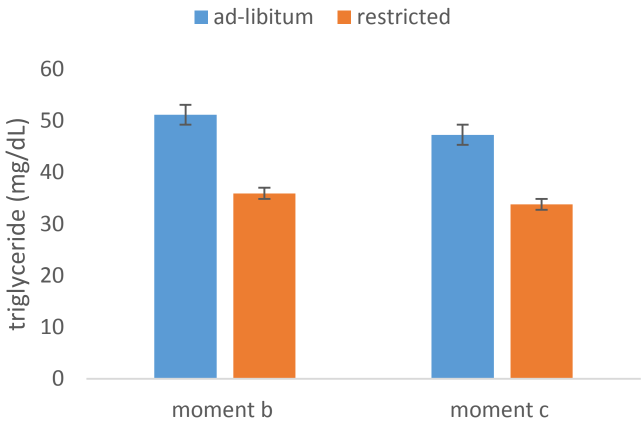

Figure 34 Triglyceride levels in animals fed ad-libitum and in those fed restricted at the middle (B) and at the finish (C).

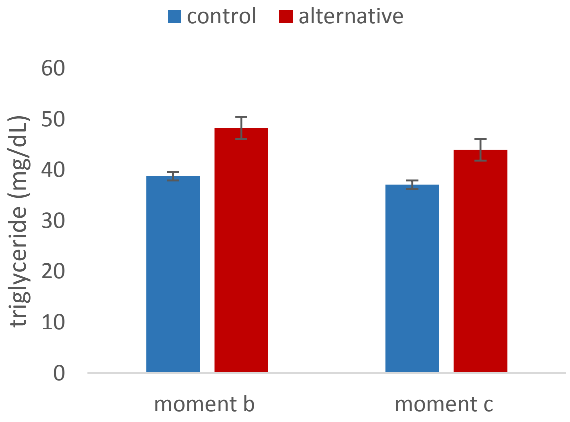

Figure 35 Triglyceride levels at the final sampling (C) in animals fed with the control diet and in those fed with the alternative one

LDL didn´t show any difference during the study when comparing between A and C. Although there has been no difference between A and B, a decrease in C was noticed

(P=0.0052), (figure 36). There was difference between regimen in B (P = 0.0266) and C (P = 0.0067): animals fed ad-libitum presented higher levels than those fed restricted (figure 37).

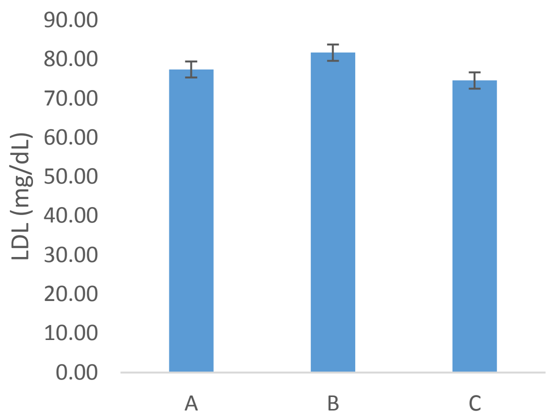

Figure 36 LDL levels during the three samples: at the beginning (A), the middle (B) and the finish (C)

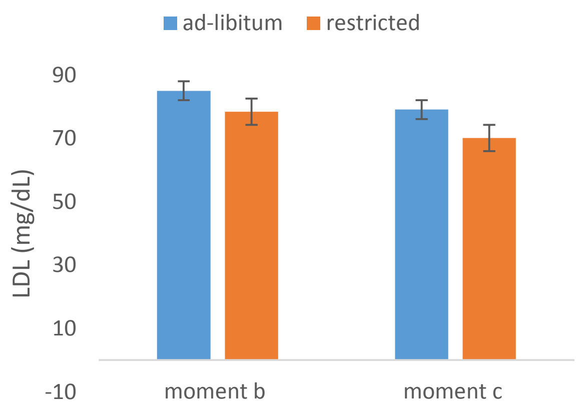

Figure 37 LDL levels in pigs fed ad-libitum and in those fed restricted in moment B and C

HDL showed an overall increase (P < 0.0001) during the study: it increased in B (P < 0.0001) when compared with A but no difference was found between B and C (figure 38). It was found effects of diets and regimen in B and C: animals fed ad-libitum presented higher levels in B (P = 0.0143) and C (P = 0.0003) than those fed restricted (figure 39). In addition, animals fed with the alternative diet had higher HDL levels than those fed with the control diet in B (P = 0.0003) and C (P = 0.0002), (figure 40).

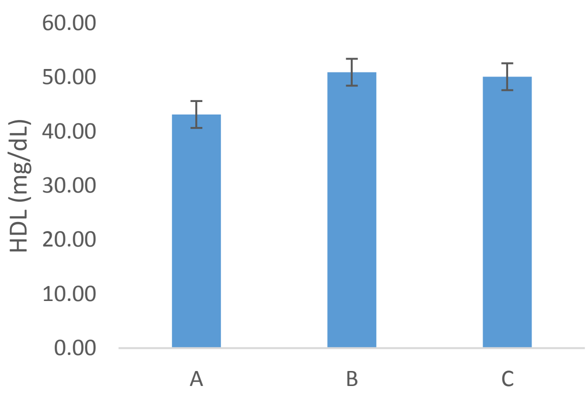

Figure 38 HDL levels during the three samples: at the beginning (A), the middle (B) and the finish (C)

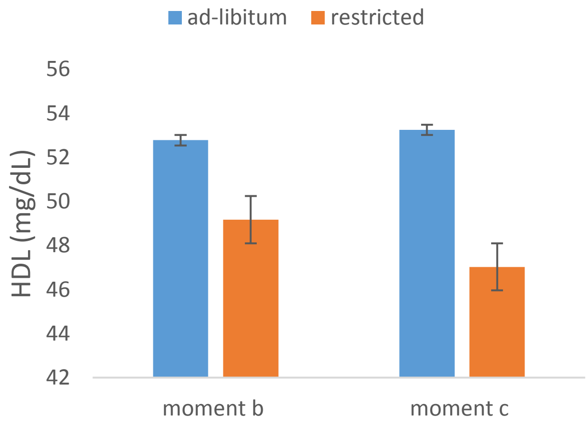

Figure 39 HDL levels in pigs fed ad-libitum and those fed restricted in moment B and C

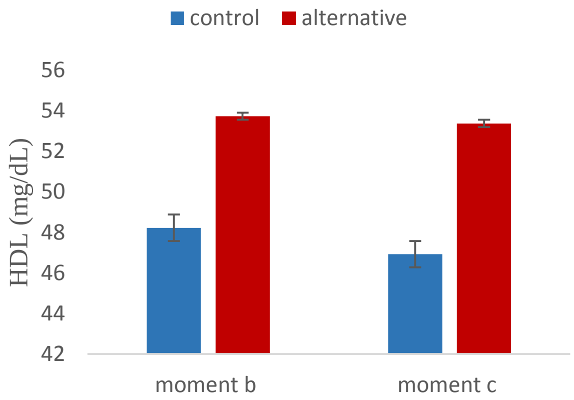

Figure 40 HDL levels in pigs fed with the control diet and those fed with the alternative one in moment B and C

Carcass traits

The effect of restriction on meat quality traits is presented in table 5. The pigs fed ad-libitum had greater live weight, carcass weight and fat thickness (P < 0.0001) than animals fed restricted. However, ad-libitum fed pigs had lower dressing percentage (P = 0.029), percentage of lean (P < 0.0001), loin thickness (P = 0.0247) and a* value (P = 0.0149) than those fed restricted. In addition, no difference was seen in PH and electrical conductivity in longissimus dorsi (LD) and semimembranosus muscle (SM), L*, b* and intramuscular fat when comparing between the regimens applied.

Table 5 Meat quality traits difference between the two regimens applied

	Ad - libitum	**Restricted**	**P value**
live weight	136,92	123,68	<.0001
carcass weight	100,92	92,13	<.0001
dressing percentage	73,72	74,48	0,029
% of lean	23,85	33,09	<.0001
fat thickness	49,32	40,34	<.0001
loin thickness	28,93	32,03	0,0247
PHuSM	5,63	5,56	0,166
ECuSM	4,13	4,40	0,227
PHuLD	5,62	5,62	0,9628
ECuLD	3,21	3,40	0,0768
L*	48,68	48,94	0,6042
a*	7,68	8,43	0,0149
b*	0,08	-0,34	0,0867
intramuscular fat	5,19	4,58	0,1207

The effect of restriction on FA composition of subcutaneous fat is presented in table 6. Pigs fed ad-libitum had lower linolenic acid (P = 0.0421) and n3 (P = 0.0409) than restricted pigs while no significant difference was seen in the other traits measured.

Table 6 Fatty acids composition of subcutaneous fat in animals fed ad-libitum and restricted

	Ad-libitum	**Restricted**	**P value**
C16:0 (palmitic)	18,54	18,25	0,6146
C16:1 n9	0,33	0,34	0,3282

C17:0 (margaric)	0,18	0,29	0,0527
C18:0 (stearic)	10,83	10,59	0,4984
C18:1 n9 (oleic)	50,16	49,66	0,2591
C18:2 n6 (linoleic)	11,35	11,80	0,0819
C18:3n3 (linolenic)	0,73	0,77	0,0421
SFA	30,99	30,55	0,5067
MUFA	56,03	55,95	0,8778
PUFA	12,95	13,48	0,0682
SFA/UFA	0,45	0,44	0,5467
MUFA/PUFA	4,35	4,20	0,0834
n6	11,54	12,00	0,0818
n3	0,87	0,91	0,0409
n6/n3	13,49	13,44	0,7379

The effect of adding fat in the alternative diet on meat quality traits is presented in table 7. Only dressing percentage differed between diets (P = 0.0187) while no significant difference was seen in the other traits measured: animals fed with the alternative diet presented higher dressing percentage than animals fed with the control diet.

Table 7 Meat quality traits in animals fed with the control and alternative diet

	Control	**alternative**	**P value**
live weight	130,90	129,69	0,6403
carcass weight	96,46	96,60	0,9443
dressing percentage	73,69	74,51	0,0187
% of lean	28,33	28,60	0,8695
fat thickness	45,16	44,49	0,7095
loin thickness	30,62	30,34	0,8349
PHuSM	5,58	5,61	0,4961
ECuSM	4,07	4,46	0,0812
PHuLD	5,62	5,62	0,931
ECuLD	3,34	3,26	0,4489
L*	48,60	49,02	0,38
a*	8,18	7,93	0,4014
b*	-0,12	-0,14	0,9259
intramuscular fat	4,63	5,14	0,1849

The effect of adding fat in the alternative diet on FA composition of subcutaneous fat is presented in table 8. Animals fed with the control diet presented higher levels of palmitic (P = 0.0014), margaric (P = 0.0451), PUFA (P = 0.0061), n6 (P = 0.0446), stearic, linolenic, SFA, SFA/UFA and n3 (P < 0.0001) than animals fed with the alternative diet. While, lower levels of palmitoleic, oleic, MUFA, MUFA/PUFA and n6/n3 (P < 0.0001) were found when animals fed with the control diet were compared with those fed with the alternative diet.

Table 8 Fatty acid composition of subcutaneous fat in animals fed with the control and alternative diet

	Control	**alternative**	**P value**
C16:0 (palmitic)	19,33	17,46	0,0014
C16:1 n9 (Palmitoleic)	0,29	0,38	<.0001
C17:0 (margaric)	0,29	0,18	0,0451
C18:0 (stearic)	11,82	9,59	<.0001
C18:1 n9 (oleic)	46,47	53,36	<.0001
C18:2 n6 (linoleic)	11,83	11,32	0,0498
C18:3n3 (linolenic)	0,84	0,66	<.0001
SFA	32,96	28,58	<.0001
MUFA	53,38	58,59	<.0001
PUFA	13,62	12,82	0,0061
SFA/UFA	0,49	0,40	<.0001
MUFA/PUFA	3,95	4,60	<.0001
n6	12,04	11,50	0,0446
n3	1,00	0,77	<.0001
n6/n3	12,04	14,89	<.0001

Behavioral results

Dirtiness

The pen dirtiness wasn´t affected by the regimen, diet or period. The dirtiness differed between zones within the pen ($P < 0.0001$): the zone 5 being the dirtiest (figure 41). Animal dirtiness was affected by the regimen ($P = 0.0007$), the diet ($P < 0.0001$) and the period ($P < 0.0001$): animals fed ad-libitum were dirtier than those fed restricted were (fig 43) and animals fed with the control diet were dirtier than those fed with the alternative one (fig 44), In addition, during the early periods, especially period 2, the pigs were dirtier than during the later periods (fig 45).

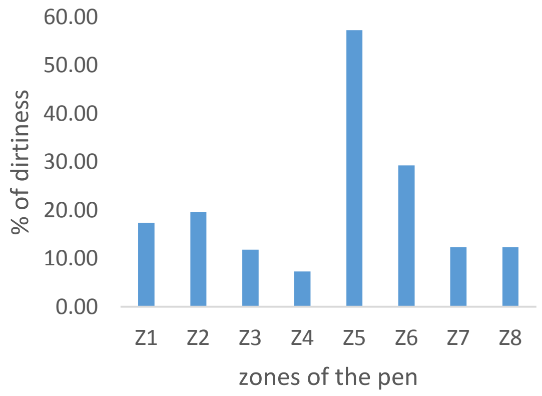

Figure 41 Dirtiness percentage in different zones of the pen

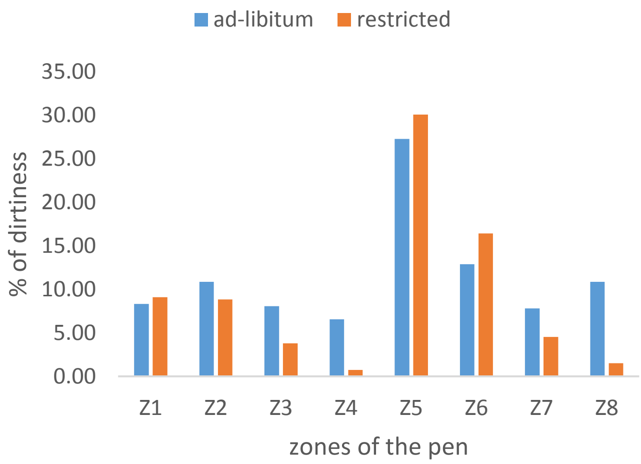

Figure 42 Dirtiness percentage in different zones of the pens according to the regimens applied (ad-libitum and restricted)

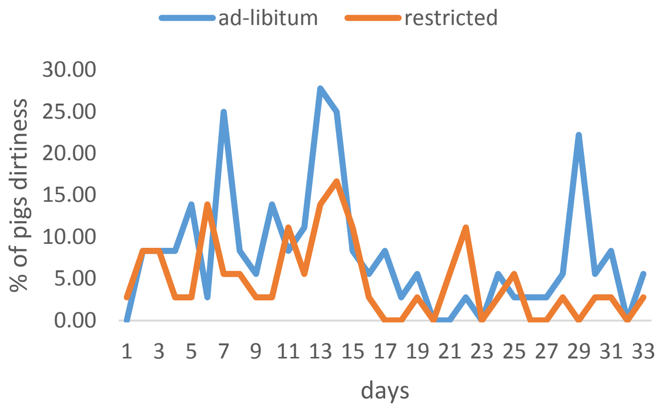

Figure 43 Percentage of pigs´ dirtiness in the two regimens applied (ad-libitum and restricted) along the study

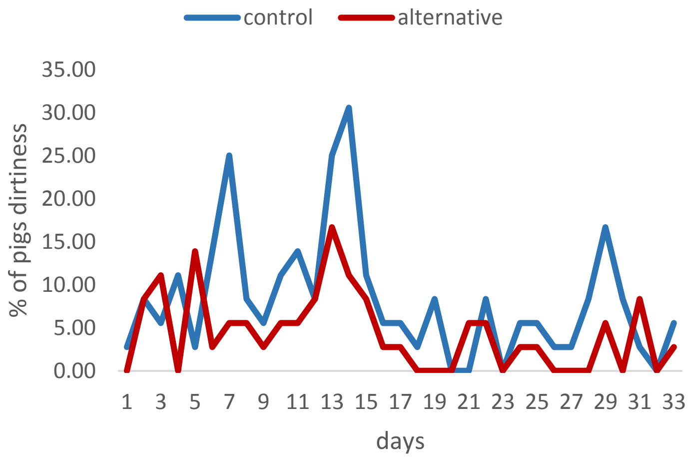

Figure 44 Percentage of pigs´ dirtiness according to the diet fed (control and alternative) along the study

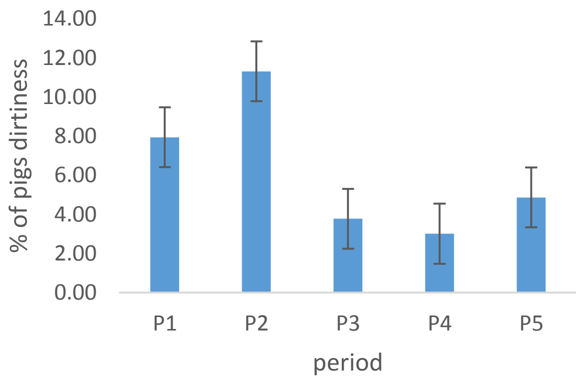

Figure 45 Percentage of pigs´ dirtiness during the different periods of the study

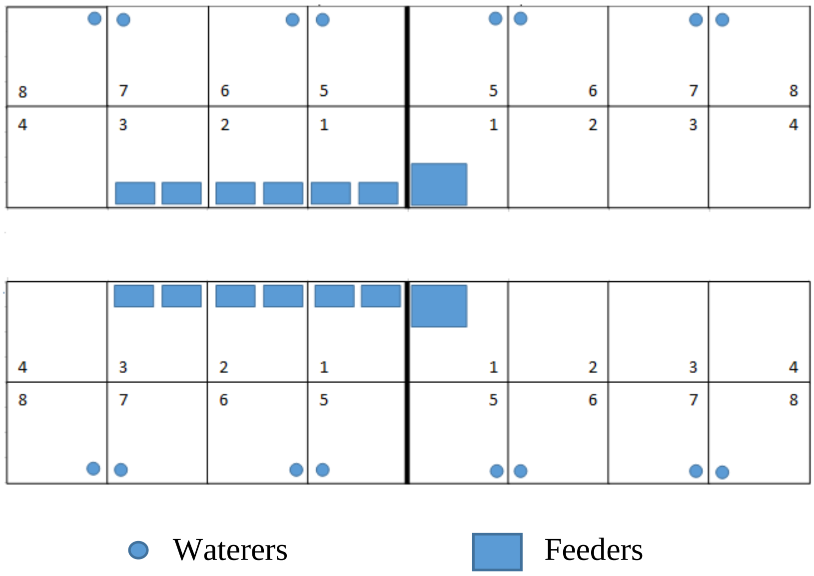

Figure 46 Scheme showing the numbering of the different pen zones

Lying position

There were zones that were preferred to lie laterally like zone 4 (P < 0.0001) and sternly like zone 8 (P < 0.0001) (figure 47). Lying laterally occurred mainly at noon (P < 0.0001), while lying sternly was mainly during the afternoon (P = 0.0179). In addition, it was seen that pigs fed ad-libitum were lying on their lateral side more than those fed restricted did (P < 0.0001) (figure 48).

Lying laterally was also affected by the interaction between zone and regimen (P < 0.0001) where the pigs fed ad-libitum preferred all zones with a very low use of zone 1 to lie in, while pigs fed restricted preferred zone 1. In addition, lying sternly was affected by interactions between diet and moment (P = 0.0341): animals fed with the control diet were lying sternly mainly during the afternoon while those fed with the alternative diet were lying during the morning (fig 49). Moreover, it was affected by the interaction between regimen and moment (P = 0.0492): animals fed ad-libitum preferred to lie during the afternoon while those fed restricted were lying during the morning (fig 50).

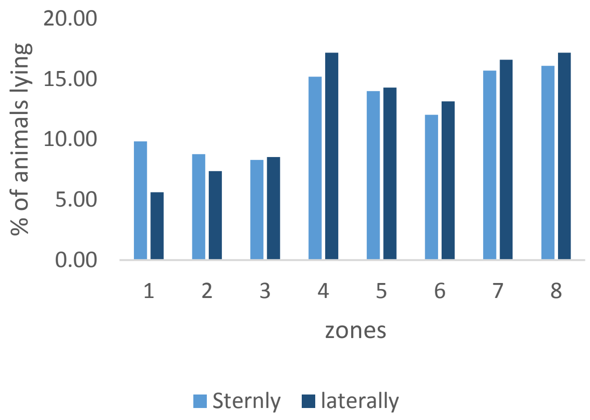

Figure 47 Percentage of pigs lying laterally and sternly in the different zones

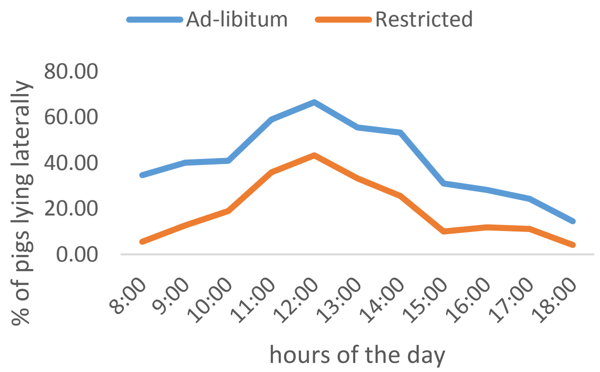

Figure 48 Percentage of pigs lying laterally according to the regimens applied

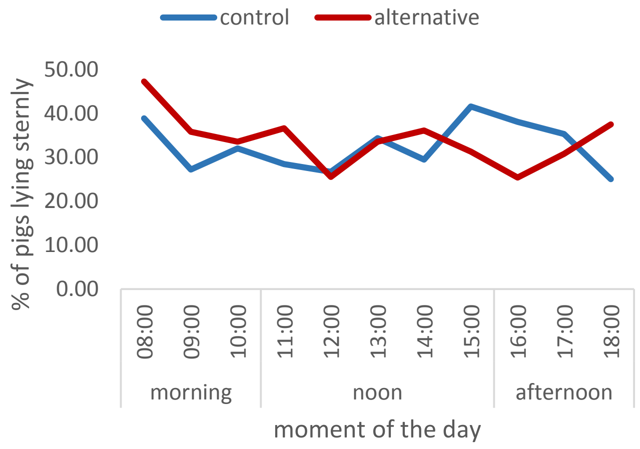

Figure 49 Percentage of pigs lying sternly during the different moments of the day and according to the diet applied (control and alternative diet)

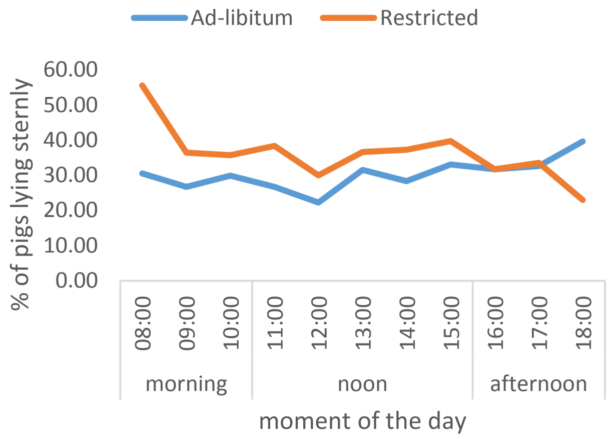

Figure 50 Percentage of pigs lying sternly during the different moments of the day and according to the regimens applied (ad-libitum and restricted)

Drinking behavior

Although no difference between diets was observed on the drinking behavior, it was noticed that pigs fed restricted were drinking water more than those fed ad-libitum ($P < 0.0001$). In addition, interaction between moment and regimen was found ($P = 0.0267$): animals fed restricted consuming water most frequently at noon and those fed ad-libitum drinking during the afternoon (fig 51).

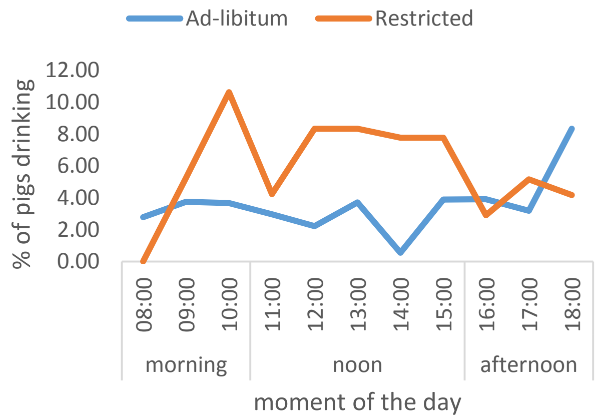

Figure 51 Percentage of pigs drinking affected by the regimens applied and the moment of the day

Positive and negative interactions

Pigs fed ad-libitum had higher positive interactions than those fed restricted ($P = 0.0178$) (fig 52). It was found that there was a high frequency of positive interactions during the morning (P = 0.0060) and of negative interactions during the afternoon (P = 0.0035) (fig 53). The positive interactions differed according to the zone and regimen applied. For example, they were abundant in zones 7 and 8 (P = 0.0012) and pigs fed ad-libitum interacted positively most frequently in zone 2, 6 and 7 while those fed restricted interacted in zone 1, 4 and 8. In addition, it was seen an interaction between zone and moment (P = 0.0153): during the morning, the higher rate of positive interactions was found in zones 2, 7 and 8, while during the afternoon, it was found in zones 3, 4, 5 and 6.

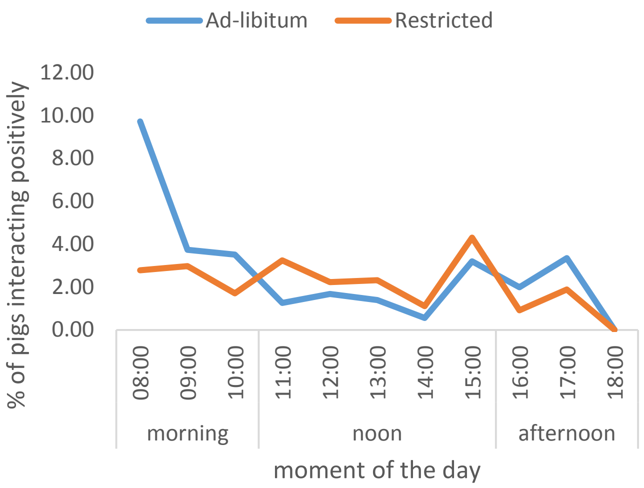

Figure 52 Percentage of pigs interacting positively according to the regimen applied

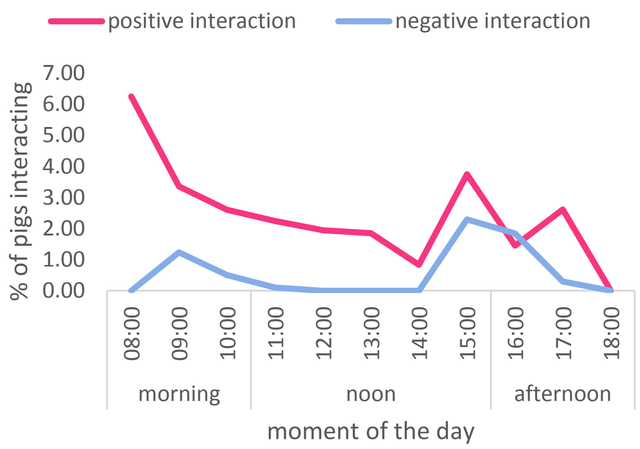

Figure 53 Pigs social behavior during the different moments of the day

Exploring behavior

Exploring the pen by the pigs differed between regimens (P < 0.0001): pigs fed restricted explored the pen more than those fed ad-libitum. In addition, the exploring behavior differed between the pens zones (P < 0.0001): pigs explored zones 2 and 3 more than the other zones and mostly during the afternoon (P < 0.0001) (figure 54 & 55).

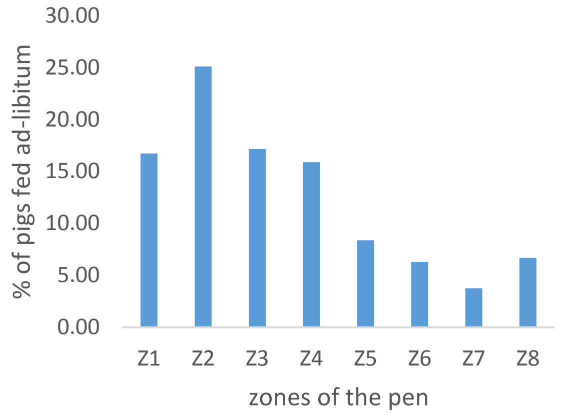

Figure 54 Percentage of pigs fed ad-libitum exploring the different pen zones

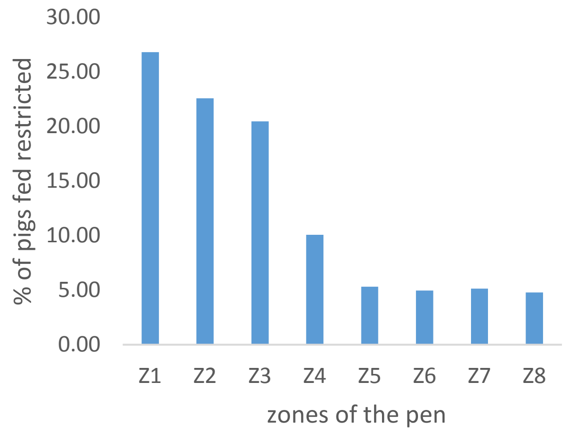

Figure 55 Percentage of pigs fed restricted exploring the different pen zones

<u>Focal sampling</u>

Hierarchy

The pigs' hierarchies were evaluated based on the social contacts between the animals, in which it was given to the pigs +1 if they win the social interaction (basically a displacement), -1 if lost and 0 if there was no clear winner or loser. Then all the animals within each pen was classified according to its score that ranged from +200 to -67 when all the animals observed are considered. Within each pen the hierarchy (from 1 to 6) was determined according to this score, the pig number 1 being the one with the highest score and the pig with number 6 being the one with the lowest. These values of hierarchy were then compared with other variables, such as feeding time, changing position and time until the first stop of eating.

Feeding time

In pigs fed restricted, in which the behavior of feeding can be observed two times per day during 29 days of the experiment, the average time to finish the meal was 1321 seconds, and on average the animals feeding were interrupted 4 times. However, a 30.47% of the times, pigs were not interrupted. On the other hand, there was animals that remained stable in their positions, while others were displaced or moved to other positions. It was observed that the position of the feeder (from 1 to 6, figure 56) had an important effect on this. In fact, pigs took the first stop earlier than the others did when they were eating in feeders 5 and 6, while pigs eating in feeders 3 and 4 took the first stop the last. These last positions were preferred by pigs in the last position of the hierarchy, but not by those in the highest (table 9).

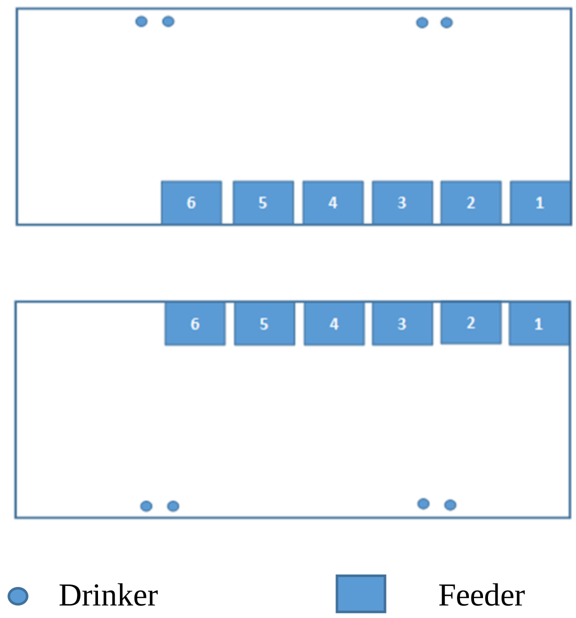

Figure 56 Numbering of feeders of restricted fed pigs

Table 9 Table resuming the animal hierarchy, its position in feeders, repetition rate and number of changing the feeder

pen	animal	hierarchy	most frequent position in feeder	repetition rate (%)	changing the feeder
13	A	5	2	27,59	2
13	B	3	1	26,32	4
13	G	2	6	31,03	3
13	N	6	3	24,14	4
13	R	4	5	25,86	2
13	V	1	2	22,41	2
14	A	2	1	31,58	3
14	B	5	2	35,09	4
14	G	6	5	29,82	3
14	N	3	4	24,56	2
14	R	1	6	28,07	6
14	V	4	4	54,39	4
23	A	2	1	29,82	6
23	B	1	1	51,79	4
23	G	4	6	35,71	3
23	N	6	3	24,56	6
23	R	3	3	36,84	4
23	V	5	4	55,36	3
24	A	6	3 & 4	26,32	3
24	B	1	4	24,56	3
24	G	2	6	42,86	3
24	N	3	5	31,58	3
24	R	5	2	24,56	4
24	V	4	1	56,14	1
33	A	2	4	65,45	1
33	B	6	3 & 5	30,91	4
33	G	1	6	80	3
33	N	3	2	49,09	4
33	R	5	3 & 5	21,82	3
33	V	4	1	52,73	3
34	A	4	1	32,14	2
34	B	6	4	51,79	3
34	G	1	1	33,93	4

34	N	3	5	26,79	2
34	R	2	3	28,57	4
34	V	5	2	35,71	3

Furthermore, the pigs who didn´t change their feeder were the last one taking the first stop ($P < 0.0001$) and had the highest average time eating ($P < 0.05$). Although the pigs who changed a lot their feeder (> 5 times) took the first stop earlier than the others did ($P < 0.05$), they didn´t have the lowest average time eating, it was the pigs who changed their feeder from 2 to 5 times ($P < 0.05$), (figure 57).

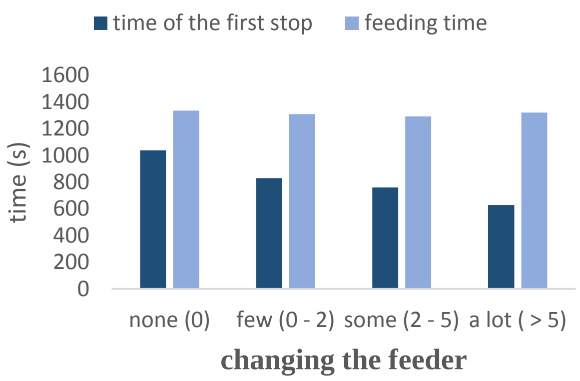

Figure 57 Time of the first stop and feeding time affected by the number of changing the feeder

At the beginning of the experiment (period 1), the pigs presented higher time eating before taking the first stop than later ($P < 0.0001$), (figure 58).

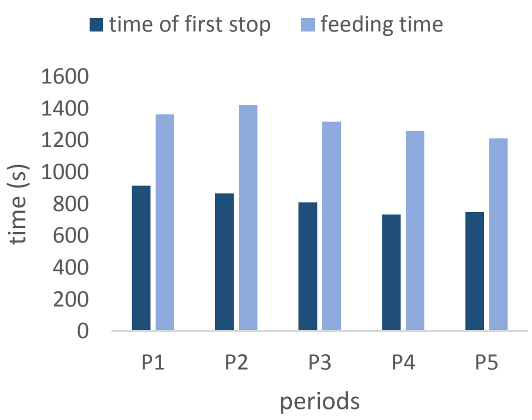

Figure 58 Time of the first stop and feeding time during the 5 periods of the experiment

Overall, Pigs with medium hierarchy presented the highest average time eating and a longer duration eating before taking the first stop than pigs with low and high hierarchy (P < 0.0001), (figure 59).

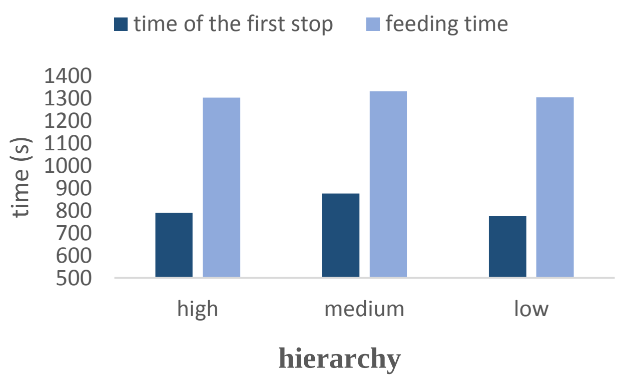

Figure 59 Time of the first stop and feeding time affected by the animal hierarchy

It was observed that pigs fed with the alternative diet had lower average time eating (P < 0.05) and took the first stop earlier than pigs fed with the control diet (P < 0.0001) as shown in figure 60.

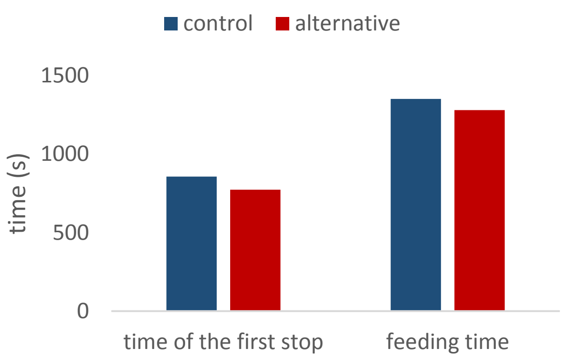

Figure 60 Time of the first stop and feeding time when using the two diets (control and alternative) during the experiment

V. Discussion

Performance

When comparing between regimens (ad-libitum vs restricted) in the growing phase, it was observed that the feed intake and average daily gain decreased in restricted in comparison to ad-libitum and the feed conversion ratio was similar in both regimens. Serrano et al (2009) reported similar results when applying a feed restriction of 18% and 28% to growing pigs (Iberian x Duroc). Daza et al (2003) also agreed with these results, but when applying a more severe restriction, an increase in FCR was observed in the restricted ones. However, Ugwu and Onuimonyi (2009) observed a slight reduction in feed conversion ratio in pigs maintained under a similar level of restriction. When compared to ad-libitum, the difference found with the present study might be explained by the fact that different breed was used (Large White x Landrace pigs) that may respond differently to the feed restriction.

As for the finishing phase, it was observed that FI and ADG decreased while FCR increased when applying a feed restriction. Although Cho et al (2006) study using animals from 50 kg to slaughter weight agreed with those of the present study concerning the feed intake and the average daily gain, he showed no significant difference in the feed conversion ratio between animals fed restricted and those fed ad-libitum.

The difference seen in FCR between growing and finishing phase might be related to one of these three factors or the combination between them: 1) Effect of animal´s metabolism: when the animal reaches the finishing phase, lean deposition decreases and fat deposition increases leading to an increased FCR (Piao et al., 2004). 2) Pig´s age can affect its performance especially in the high ambient temperature condition applied in this study. As pig is heavier, his capability to adapt to heat stress is lower than a leaner pig (Collin et al., 2001), due to the lower surface in comparison to the body mass when the pig is bigger (as lower the ratio surface/body mass more difficult for the animal for dissipating heat). 3) The change of the diet: this experiment was held during the growing-finishing period. When the diet was changed from grower to finisher, the pigs presented different ADG being higher before changing the diet than after (0.910 vs 0.780 kg/day).

When comparing between diets (control vs alternative) in the growing phase, it was observed that the feed intake reduced when fat is added. Although the results of Baudon et al (2003) and Myer and Combs (1991) were in accordance with those of this study, no significant difference of adding fat to the diet was observed in De la Llata et al (2001), Brooke et al (2015) nor Overland et al (1999) experiments. On the other hand, when comparing the effect on the average daily gain, only the results of Myer and Combs (1991) agreed with the results obtained in this study showing that there was no effect of adding fat on the ADG. On the other hand, the reduction in Feed conversion ratio is already described in most of the previous cites, except in the case of Brooke et al (2015) that observed no changes in the FCR when adding fat. In any case, only Myer and Combs (1991) study found the combination of the same results of FI, ADG and FCR obtained by this study, showing that the feed intake decreased, ADG was not affected and the feed conversion ratio was improved.

As for the finishing period, it was observed that feed intake decreased when adding fat to the diet, which is in accordance with the results of Baudon et al (2003), De la Llata et al (2001), and Myer and Combs (1991) and not in accordance with Brooke et al (2015) and Overland et al (1999). This comparison differed when observing the effect on the average daily gain. It was observed in this study no significant effect on the average daily gain, which is in accordance with De la Llata et al (2001), Brooke et al (2015) and Myer and combs (1991) results, while Campbell (2005), Baudon et al (2003) and Overland et al (1991) showed that ADG was improved. In addition, when comparing the feed conversion ratio, Brooke et al (2015) was the only author that agreed with the results of this study showing that it was not altered. However, no one of the previous studies agreed with the combination of effects found on FI, ADG and FCR after supplementing fat in finishing pigs.

The difference found between the present study and other previous studies could be explained by several factors, such as genetics and environmental temperatures. For example, Brooke et al (2015) experiment was held in a normal ambient temperature and using Landrace x Large White pigs while this study was held in a high ambient temperature and using Iberian pigs (50% Iberian x 50% Duroc). In addition, Overland et al (1999), who found that there was no significant effect on the feed intake, an increase in average daily gain and a decrease in the feed conversion ratio, used a higher fat supplementation (10%) in a semi ad-libitum feeding regime twice daily.

Coffey (1999) described that pigs consume feed in order to meet their energy requirements and as fat is high-energy ingredient, when increasing it in the diet, the animal´s feed intake will decrease consuming the same amount of energy required. This could explain the effects of the decrease in feed intake in both growing and finishing phase in the present study.

The response of feed conversion ratio differed between the two phases of growth (growing and finishing) when using the alternative diet. The difference seen in FCR between growing and finishing phase might be related to one of these three factors or the combination between them: 1) Effect of animal´s metabolism: when the animal reaches the finishing phase, lean deposition decreases and fat deposition increases leading to an increased FCR (Piao et al., 2004). 2) Pig´s age can affect its performance especially in the high ambient temperature condition applied in this study. As pig is heavier, his capability to adapt to heat stress is lower than a leaner pig (Collin et al., 2001), due to the lower surface in comparison to the body mass when the pig is bigger (as lower the ratio surface/body mass more difficult for the animal for dissipating heat). 3) The change of the diet: this experiment was held during the growing-finishing period. When the diet was changed from grower to finisher, the pigs presented different ADG being higher before changing the diet than after (0.910 vs 0.780 kg/day). The results highlight the importance of this kind of studies in heavy breeds (animals slaughtered in high body weights) in which the finishing period is extended in comparison to animals slaughtered around the 100 kg of body weight. As in this study, it was obtained, in general, a better agreement with previous literature during the growing phase than during the finishing phase.

<u>Stress</u>

In the present study, the hematocrit levels were higher at the end than at the beginning of the experiment. These findings are in agreement with those of Sreedhar et al (2013) who observed elevated hematocrit levels in pure Sahiwal and Jersey x Sahiwal crossbred cows subjected to high environmental temperature conditions. The explanation in their case is that this high environmental temperature produces a dehydration in the animals and as consequence, it can be found a hemoconcentration. In consequence, although in our study water was provided ad-libitum, the effect of the environmental temperatures cannot be discarded. In fact, other measures showed some signs of stress on the animals. For instance, leucocytes count, although decreased from the beginning to the end of the study, was by far higher than the normal range for this species during all the study 10000 to 15000/µL vs the 23949 and 19151 leukocytes/µL of blood in basal and final samplings, respectively. The high value at the beginning of the study could be explained for the stress produced after transportation, mixing of animals, arrival to a new environment, etc…, but the values found later would confirm that animals were not completely free of stress during the growing and finishing period in the conditions of the study. Actually, McGlone and Pond (2002) concluded that when pigs are subjected to an active infection or stress, leucocyte level increases commonly two folds. Therefore, the values found in hematocrit and leukocytes would confirm that the heat treatment applied to the animals was enough to produce some stress. On the other hand, although the basal sample of blood was taken to the animals 10 days after the arrival to the new facilities, the results suggest that more time could be needed in this type of animals subjected to a so long transportation (around 800 km from the origin to the destination). In addition, this would explain as well the lack of a clear effect on the neutrophil/lymphocyte ratio, being higher at the beginning than at the end of the project. Therefore, at the moment, the only valid conclusions in terms of stress is that there are signs showing that indeed the thermal treatment was producing some stress to the animals, but not enough to be comparable to the transport and adaptation conditions at the beginning of the study, even when samples were taken 10 days later.

According to the results found for hematocrit and leukocytes count, it was found an increase in creatinine levels when the final sample was compared with the basal one. Sreedhar et al (2013) studying Sahiwal and Jersey x Sahiwal cows in tropical environments found the same effect, suggesting as an explanation that the blood flow was reduced to the intestinal organs because of heat stress and as a result, kidneys are unable to function properly, which influence the rate of creatinine excretion. This would produce high levels of this metabolite in the blood (Sreedhar et al., 2013). Scharaf et al., (2010), described that urea increases as well due to dehydration. However, in the present study this increase in urea was seen in the second sample (after the growing period) and not at the end of the study. Therefore, an effect of the diet in both phases cannot be discarded.

In comparison to the basal levels, ALT increased at the end of the study, but not AST. ALT is related to hepatocytes injury or alteration in liver membrane permeability (Price and Albert, 1979; Ramaiah, 2007), while AST is not specific to hepatocellular injury and it can reveal disorders in other organs and tissues, particularly striated muscle (ASCP, 2003). ALT serum activity might be affected by other factors not related with hepatic necrosis, such as cholesterol levels (Kim et al., 2008). Accordingly, in the present study it was found an increase in both, cholesterol and ALT.

On the other hand, the effects found in AST were not very consistent along the study, as an increase was observed in the growing phase in comparison to the basal value and a decrease in the finishing phase when compared with the same value, so the final result, as mentioned above, is a lack of signification for the whole study.

<u>Diet effect on blood and carcass quality</u>

Pigs fed with the high fat diet showed high serum cholesterol and triglycerides levels than control pigs. These results agree with those of Eisinger et al (2014) studying the effect of a high fat diet on circulating lipid species in mice. Although high levels of cholesterol were found, no differences were seen in LDL levels while HDL was higher in fat diet than control diet. HDL, considered as the good cholesterol, collect extra cholesterol in the body and carry it back into the liver in order to eliminate it when it is not needed, which means that the high levels obtained in this experiment could be considered as a good sign. In another hand, LDL, the bad cholesterol, didn´t show any differences in its levels when the final value is compared with the basal one, which show that increasing the diet content in fat didn´t show bad consequences on serum lipid content of the animals.

Overland et al (1999) observed a higher live weight in a high fat diet than in control one and Baudon et al (2003) showed a higher carcass weight in supplemented fat diet in comparison with the control diet. However, in the present study it was not found a diet effect in live or carcass weight. This agrees with results of Brooke et al (2015), although in their case they did not find any effect in the dressing percentage and we did. Why the dressing percentage was higher in the alternative diet? One hypothesis could be related with the consumption of food. During the study, the pigs fed with the alternative diet had a lower feed intake than those fed with the control diet. As consequence the gut and stomach content at the time of slaughter would be reduced in the first group of animals, improving the dressing percentage. Another explanation is related with the intestinal organs weights as Bikker et al (1996) described that an increase in ADG would induce an increase in their size and in consequence a lower dressing percentage.

In addition, it was observed that the two diets applied in this study did not influence either lean percentage, fat and loin thickness, pH or the color of semimembranosus muscle. These results are in accordance with those of Sardi et al (2007) studying the effect of supplementing a 3% high oleic diet on Duroc x Large White pigs.

In the case of Sardi et al (2007), the high oleic diet resulted in a higher oleic level in terms of carcass quality. This agrees with the results of the present study, where the fat diet produced carcasses with higher oleic, lower palmitic and stearic acids than the control diet. Consequently, the alternative diet resulted in higher MUFA and lower SFA than the control diet. In addition, contrarily to the results obtained by Realini et al (2010) using a 10% added fat diet of different sources like high oleic sunflower, in the present study PUFA, C18:2 and C18:3 were higher in carcasses from pigs fed with a control diet than those fed with a high fat diet. In the present study, the fat added was a high oleic sunflower oil explaining why high oleic acid was obtained. Sardi et al (2007) described that the fatty acids profile of the subcutaneous fat reflects the fatty acids composition of the diet, which might explain the high oleic acid obtained when using high oleic sunflower oil in the diet.

Regimen effect on blood and carcass quality

Higher cholesterol, triglyceride, LDL and HDL levels were seen in animals fed ad-libitum compared to the restricted ones due to the increase in consumption of food.

Ad-libitum fed pigs had a heavier live and carcass weight than those fed restricted. However, the dressing percentage of pigs fed ad-libitum was lower than restricted animals. This can be explained by the fact that ad-libitum animals were not fasted before transportation and they were killed before the 24h after loading. In other cases, with long fasting periods, it is easy to see no differences between restricted and fasted ad-libitum fed animals (Serrano et al 2009). The difference between live and carcass weight is caused by the gut fill that may be affected by many factors such as feeding level, diet characteristics and time-off feed (Stranks et al., 1988).

Contrarily to the results obtained in this study showing that fat thickness was greater in pigs fed ad-libitum than in those fed restricted, Serrano et al (2009) showed that fat thickness was similar in both regimen ($P = 0.051$). However, loin thickness was lower in ad-libitum fed pigs than in the restricted ones, which agrees with the results of Daza et al (2006), in which Iberian pigs were restricted to 50% of ad-libitum consumption.

Economical aspects

The 36 pigs fed with the control diet consumed 2.97 kg/day and 3.58 kg/day during the growing (56 days) and finishing phase (37 days), respectively. Taking into consideration the price of the control diet (0.368 euros/kg), the total price of the control diet consumed during the study was 3958.23 euros. As for the alternative diet, the pigs consumed 2.79 kg/day and 3.21 kg/day during the growing and finishing phase, respectively. Taking into account the price of the alternative diet (0.404 euros/kg), the total price of the alternative diet consumed was 3999.74 euros. It can be seen that feeding with the alternative diet resulted in a higher price than when feeding with the control one with a difference of 41.51 euros in total (1.15 euros per animal).

As for the carcass weight of animals fed with the control diet (96.46 Kg) and those fed with the alternative one (96.60 Kg), the carcass originated from pigs fed with the alternative diet was 0.14 Kg/animal higher than the carcass originated from pigs fed with the control diet showing a difference of 5.04 Kg in total animals per diet. Considering that the price of 1 Kg of meat sold was 1.3 euros, the price of the extra weight obtained in animals fed with the alternative diet is 6.55 euros.

When comparing between prices, it can be noticed a deficit of 34.96 euros when using the alternative diet. Therefore, 1 Kg of meat originating from carcasses of pigs fed with the alternative diet costed 0.01 euros more than 1 Kg of meat originating from carcasses of pigs fed with the control diet. However, it is easy to recover this cent or even obtain some extra benefits of the animals fed with the alternative diet if we take into account the added value that could be for the consumer the higher oleic acid found. The fatty acid profile, particularly the oleic acid, of tissue lipids from Iberian pigs affects technological, sensory and nutritional quality of meat since the physical state of fat (liquid or solid) highly depends on it. For the Iberian pig fat, the oleic acid is the main contributor to fat fluidity (Ventanas et al., 2005) giving a soft and oily aspect to the fat. These features (soft and oily fat) are accepted and highly valued by the consumers of Iberian dry cured ham due to the high oleic content and the desirable flavor (Ruiz et al., 2002).

Between the 36 pigs fed ad-libitum there was 18 that were fed with the control diet and the other 18 were fed with the alternative diet during the growing (56 days) and finishing phase (37 days). Pigs fed with the control diet consumed 3.18 kg/day and 3.77 kg/day during the growing and finishing phase, respectively, and the pigs fed with the alternative diet consumed 2.98 kg/day and 3.40 kg/day during the growing and finishing phase, respectively. Considering the prices of the control (0.368 euros/Kg) and the alternative diet (0.404 euros/Kg), when feeding the pigs ad-libitum it costed in total 4231.95 euros (117.55 euros per animal).

In addition, between the 36 pigs restricted there was 18 that were fed with the control diet and the other 18 were fed with the alternative diet during the growing (56 days) and finishing phase (37 days). Pigs fed with the control diet consumed 2.76 kg/day and 3.39 kg/day during the growing and finishing phase, respectively, and the pigs fed with the alternative diet consumed 2.61 kg/day and 3.06 kg/day during the growing and finishing phase, respectively. Considering the prices of the control (0.368 euros/Kg) and the alternative diet (0.404 euros/Kg), when the pigs were fed restricted, it costed in total 3740.86 euros (103.91 euros per animal), showing a higher price when feeding ad-libitum of 491.09 euros in total than when feeding restricted (13.64 euros per animal). Furthermore, Animals fed ad-libitum showed a higher carcass weight than those fed restricted: 100.92 vs 92.13 Kg respectively, showing a higher carcass weight when feeding ad-libitum of 8.79 Kg per animal and 316.44 Kg by regimen. Considering that the price of 1 Kg of meat was sold by 1.3 euros, the price of the extra weight obtained in pigs fed ad-libitum is 411.37 euros.

When comparing between prices, it can be noticed a deficit in carcasses originating from pigs fed ad-libitum of 79.72 euros. Therefore, 1Kg of meat originating from carcasses of pigs fed ad-libitum costed 0.02 euros more than 1 Kg of meat originating from carcasses of pigs fed restricted. In consequence, it can be observed that, when the pigs were fed restricted, they produced a slight benefit compared to the same animals produced ad-libitum if the number of days of the growing-finishing period are only considered. Of course, if the slaughterhouse asks for a specific weight, it can take longer time to obtain it with a restricted than ad-libitum regimen and in this case the costs of maintenance of the farm (water, electricity, personnel…) would eliminate this slight advantage of the restricted regimen.

Behavior

In the present study, it was found that among the same pen, zone 5 was the dirtiest. It was described that pigs like to excrete in areas far than the commotion area that is most commonly near to the feeders, and to lie away from wet areas like where the waterers were placed, for that reason pigs tend to urinate, defecate and drink in the same area (Fritschen., 1975). Furthermore, Hacker et al (1994) reported that, in pens with open partition, where the pigs can see and directly communicate with neighboring pigs, they tend to excrete along with these opening partitions due to the marking behavior, thus indicating to the neighbors their territorial limits. The results of the present experiment agree with those of Fritschen (1975) and Hacker et al (1994) previously cited, showing that pigs tended to excrete in zone 5 more than the other zones containing a waterer and sited near to an opening partition.

An elevated dirtiness in pigs was seen during the study showing that pigs were lying and wallowing in the excretion area. Aarnink et al (2001) reported that this behavior is an indication that pigs were suffering thermal discomfort. In addition, Huynh et al (2005) described that in a high ambient temperature, pigs having a limited capacity to sweet adapt a wallowing behavior in the dunging area in order to cover their body with a fresh layer to increase the evaporative heat loss leading to an elevated dirtiness. In addition, it was observed that pigs fed with the control diet were dirtier than those fed with the alternative one and pigs fed ad-libitum were dirtier than those fed restricted.

In terms of dirtiness, two initial and contradictory hypotheses were tested. The first one it was that the animals fed restricted would have the floor pens dirtiest than the animals fed ad-libitum due to the lower space in this pen for the allocation of the 6 feeders. The second, that animals fed ad-libitum, with a higher consumption of feed would have too a higher level of excretion so the floor would be dirtiest than in the restricted ones. However, although the animals that were eating more (ad-libitum regimen and control diet) were the dirtiest, there was no effect of the treatments (diets and regimens) on the floor dirtiness, so this hypothesis cannot entirely be confirmed or rejected.

During the early periods of the study, especially in period 2, pigs presented a higher dirtiness than in later periods. The high dirtiness at the beginning could explain that pigs were more stressed by heat at the beginning, being introduced to a new ambient temperature. Afterwards, in the later periods, the dirtiness decreased indicating that after a certain time, pigs were more adapted to heat stress reducing their wallowing behavior.

In addition, it was found that the pigs lied laterally mainly at noon when the temperature was increased to 30^0C for five hours, and sternly most frequently during the afternoon and morning when the temperature was maintained at 25^0C. Huynh et al (2005) explained that lying on the lateral side is a strategy adapted by the pigs to dissipate the increased body heat brought about by the increased ambient temperature by increasing the body contact with a cooler surface, the floor. In addition, it was shown that pigs fed ad-libitum were sleeping laterally more than those fed restricted, this could be a sign of a higher necessity for losing heat in the pigs fed ad-libitum than in pigs fed restricted. As pigs prefer to lie in zones beyond the active zone that is mainly the feeder zone (zone 1), pigs fed ad-libitum were sleeping in all zones but in zones 1 in a very low frequency. However, as the feeders were used only during the meals in the restricted ones (and all of them at the same time), it was observed a higher use of this zone (zone 1) in these animals compared to ad-libitum animals, confirming the importance of the activity in the different areas of the pen when pigs selected the resting area. The model of pigs fed ad-libitum is closer to what is expected under normal conditions than the model of restricted pigs: pigs fed ad-libitum were sleeping sternly mainly during the afternoon that might be considered as resting period of the day. Conversely, the restricted pigs were sleeping sternly mainly during the morning. This could be explained by the fact that the feed was given at 16:00 during the afternoon, which was more appropriate to the student schedule but not to the pigs affecting consequently the resting hours of pigs fed restricted.

A number of factors including the pen design, location of feeder and drinker, and environmental conditions determine the lying zone of the animal (Costa et al., 2004). In the present study, it was observed that pigs preferred to lie laterally in zone 4 and sternly in zone 8. Both zones are more far away from the feeder so the most suitable areas for resting but the results show that zone 4 is probably warmer than zone 8.

In the present study, the drinking behavior differed between regimens: pigs fed restricted were drinking more than those fed ad-libitum. One possible explanation is that pigs fed restricted were fed always within the observation period while the pigs fed ad-libitum could take feed in or out of the observation period. On the other hand, Fraser (1984) described that pigs drink water mainly at mealtime, which is in accordance with the results found in ad-libitum pigs, as drinking behavior coincided with feeding behavior. However, this was not the case in restricted animals, as they were eating at 9:00 and 16:00, and their drinking behavior was at noon. One possible explanation is that animals were trying to avoid drinking during the mealtime due to an increase of competition for the drinker with a discoordination between feeding and drinking in a restricted regimen. However, this need to be further studied.

The moment of the day where the social and exploring behaviors were low was at noon. Debrecéni et al (2014) reported that during the high ambient temperature, pigs are inactive and tend to lay and rest more, which would explain the reduced activity at noon. On the other hand, the positive interactions were abundant during the morning, while the negative were during the afternoon. Although the diet didn´t have an effect on the social behavior of the pigs, the regimen did and different activities were observed throughout the day within each zone of the pen depending of the treatment regimen.

The pigs fed restricted were exploring in a higher frequency than those fed ad-libitum and mainly in zones where the feeders were placed (zones 1, 2 and 3). The pigs fed restricted might be exploring in the feeding areas to search for additional feed that might be left in the feeders or on the floor.

In general, with 6 feeders for the 6 animals, each one having its own feeder, it was observed two important things. In the first place, animals were rarely able to complete a meal without an interruption. Thus, although the average time to finish the meal did not reach 25 minutes, only 30% of the times the animals managed to finish the meal without being disturbed. At the same time, the initial fidelity to the same feeder was relatively low (the initial fidelity is referred to the feeder in which the pigs began to eat before being displaced for the first time), since in the majority of the animals it oscillated between 22.4 and 36%. On another hand, although there was a decreasing pattern of the hierarchy and the average fidelity to the feeders (of all, the first and second in the hierarchy (the most dominants in each pen) had a fidelity to the feeder of 38.57%, while the third and fourth (middle position in the pen) had a fidelity of 36.12% and the fifth and sixth (the most subordinates) had a fidelity of 32.30%), there was a lot of variability between pens. For example, the most dominant of the pen 33 had an 80% of fidelity, and the most dominant of the pen 13 had a fidelity of only 22.4%.

On the other hand, it was interesting to see how the highest animals in the hierarchy tended to be more faithful to the extreme positions of the feeders, whereas the lower animals (with a greater tendency to lose the confrontations) tended to choose the most central feeders as starting position, where the first interruption occurred later. Thus, in a multi-feeder system, it seems that the most subordinates will have to eat in the central positions and the most dominant in the peripheries, where there were more interactions. In this sense, it seems that the most competitive positions resulted more interesting for the animals that usually win the fighting than for the animals that usually lose them. A possible practical application of this phenomenon would be the possibility to provide some supplements to the subordinate animals of a pen by placing them in the central feeders and making sure that they are consumed before the first stop, which on average would occur at 12 minutes.

Another interesting result is that the fidelity to the feeder did not increase with time, but rather the opposite, because as the study advanced, the time to the first interruption of food intake was shortened, probably by a greater willingness of the more dominant of the pen to access the other animals´ food.

Finally, the fact that the animals ate less kg of feed of the alternative diet than of the control diet also resulted in reduced total time observed eating even in an earlier appearance of the first stop.

Definitely, the behavioral studies indicated that the position of the feeder is important for the animals, but not enough to be able to ensure that an individual will always occupy the same feeder. However, in general, it can be expected that the subordinate animals will start in more central positions before being interrupted and this allows them to make the first feeding a little longer.

VI. Conclusions

- Although no differences were found in the final weight and average daily gain when animals were fed with any of both diets, the consumption and consequently the feed conversion ratio were lower in the alternative diet than in the control diet.
- Although no differences were found in the live and carcass weight, the dressing percentage was higher in pigs fed with the alternative diet than in pigs fed with the control diet.
- Overall, the costs of both diets were similar, although the final product differed between diets.
- The alternative diet gave a product rich in oleic acid and that could be more attractive for the consumer.
- Pigs fed with the control diet were dirtier than pigs fed with the alternative diet showing that they were suffering from heat stress more than the pigs fed with the alternative diet.
- Pigs fed restricted were less sensible to the heat stress than pigs fed ad-libitum that were dirtier and slept laterally more than pigs fed restricted.
- In restricted animals fed with a multi-feeder system, the dominant and the subordinate animals occupy initially different positions, the subordinates preferring the central positions.

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