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Universitat Autònoma de Barcelona

MANAGEMENT AND FEEDING STRATEGIES IN YOUNG HOLSTEIN BULLS FED HIGH-CONCENTRATE DIETS

A

THESIS

Presented to the Animal and Food Science Department of Veterinary Faculty of the Universitat Autònoma of Barcelona

In Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF VETERINARY

By

Núria Mach Casellas

Directed by

Maria Devant Guille

December 2008

FACULTAT DE VETERINÀRIA DE BARCELONA

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ALFRED FERRET QUESADA, Professor Titular del Departament de Ciència Animal i dels Aliments de la Facultat de Veterinària de la Universitat Autònoma de Barcelona

Certifiquen:

Que la memòria titulada "**Management and feeding strategies in young Holstein bulls fed high-concentrate diets** ", presentada per Núria Mach Casellas per optar al grau de Doctor en Veterinària, ha estat realitzada sota la direcció de Dra. Maria Devant Guille i, considerant-la acabada, autoritza la seva presentació per què sigui jutjada per la comissió corresponent.

I per tal que consti els efectes que corresponen, signa la present a Caldes de Montbui, 22 d'Octubre de 2008

Maria Devant Guille
Directora

Alfred Ferret Quesada
Tutor

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"-Abans d'iniciar el viatge del Doctorat pensava que potser a la tornada escriuria algunes impressions, sensacions i sentiments, i en definitiva sobre tot allò que s'ha allunyat de la *logos* protocol·lària i metòdica del sistema empíric-analític, per tractar de fixar-les en la meva biblioteca personal de records. De la mateixa manera que realitzem fotos a petits-grans llocs meravellosos i dels moments especials per després tenir la serenor i oportunitat de reviure'ls *de novo*, amb calma, amb la contemplació dels autèntics filòsofs, en aquest viatge he pres anotacions, totes elles breus, petites referències, paraules, gestos, abraçades, somriures, suficients per tenir consciència del conglomerat ontològic que m'envolta i m'ha envoltat. Quan veus amb els teus ulls, aquest conglomerat, aquesta història, aquesta vida, el passat en el present i el mateix temps el futur en el present, la bellesa, essent extraordinària, no puc menys que intentar escriure els agraïments, ni que sigui una llunyana aproximació, sense altre pretensió de servir-me a mi mateixa i tornar a disfrutar amb l'evocació i el record. Gràcies eternes a la meva família, a l'Isa Roca, a en Ferran Garcia, a la Laura Ayats, a la Núria Fuster, a l'Emma Soy, a en Luciano Gonzàlez, a l'Artemi Damaso, a en Xavier Fernàndez, a la Roser Morató, a la Cristina Iglèsias, a l'Àngela Ferrer, a en Francesc Molist, a la Núria Sànchez, a la Sílvia Chisbert a en Josep Espadamala, i a en Carles Adelantado.

Atenes, fou considerada per Plató com creació del favor i la providència del que és diví. Una ciutat per caminar. Els seus carres no tenen la *grandeur* dels carres de París, ni la monumentalitat de Roma amb la seva via Sacra, ni té tampoc el cosmopolitisme dels de Londres, gegantesc *puzzle* de races i contrasts, ni l'aire bohèmic dels carrers de Lisboa. Però sense dubte els carrers d'Atenes, els que tu m'has ensenyat, em van atrapar al·legòricament des del primer moment. Caure en aquesta xarxa tant fàcilment com difícil fou per Ulisses evitar ser atrapat per l'encant de les sirenes ha estat un plaer. Gràcies per no només regalar-me la oportunitat d'entrar a casa teva literalment, sinó d'aprendre amb tu el brindar de les *Humanitats*.

Sense parar de caminar, ja mirant a altres direccions, evitant el tràfic dels carrers i els obstacles de les voreres, de les obres continues i intermitents, dels quioscs petits i coberts de tota classe d'articles, arribo a l'*edèn*, literalment, amb totes les seves lletres, sentint que em pertany una mica, que s'obre a mi i em saluda, que em rep i m'abraça completament cobrint tots els píxels del meu cos, deixant-me fer, com pacientment Penélope s'entretingué durant segles teixint i desteixint el tapiç del temps fins la seva arribada. És que llunyana, Ítaca, m'era propera? Gràcies *ad infinitum* David -".

PUBLICATIONS AND ABSTRACTS DERIVED FROM THIS THESIS

SCIENTIFIC PUBLICATIONS:

Mach, N., M. Devant, and A. Bach. 2005. Relación entre la fermentación ruminal y las papilas ruminales en terneros jóvenes. ITEA, Vol. Extra Nº 26 (Suppl. 2): 533-535.

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Díaz, I., **N. Mach,** and J. A. García. 2004. Ácidos grasos cis y trans en la carne y productos cárnicos. Eurocarne, 130: 51-56.

Mach, N. 2004. Carne y leche enriquecida con ácidos grasos funcionales. Entre camps i animals, 49: 30-31.

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ABSTRACTS AND CONTRIBUTIONS TO CONGRESSES AND SYMPHOSIA

Mach, N., M. Devant, A. Bach, I. Díaz, M. Font i Furnols, M. A. Oliver, and J. A. García. 2005. Improvement of omega-3 fatty acid content of meat from young Holstein bulls receiving a high-concentrate diet. Journal of Animal Science, 83 (Suppl. 1): 53. ADSA/ASA Joint Meeting Congress, Cincinnati (USA).

Mach, N., M. Devant, A. Bach, and A.Velarde. 2006. Associations between animal, transportation, and slaughterhouse practices and meat pH in beef. Journal of Animal Science, 84 (Suppl.1): 114. ADSA/ASA Joint Meeting Congress, Minneapolis (USA).

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ABSTRACT

In the present thesis, strategies to increase efficiency in intensive beef production focused mainly on feeding and management practices have been studied.

The first strategy proposed to increase the efficiency of intensive beef production has been the improvement of carcass and meat quality. Indeed, a study was conducted to evaluate the effect of different pre-slaughter factors on the incidence of high ultimate pH and extreme carcass bruises, and to make proposals pertaining to management, technical, or economic decisions, which could lead to improvements on the high incidence of high ultimate meat pH and the extreme carcass bruises observed in intensive beef production systems. Unfortunately the objective *per se* was not achieved because the variability of ultimate meat pH and carcass bruises explained by these factors was very low. In consequence, the percentage of beef carcasses with high ultimate meat pH (up to 13%) and extreme bruises (up to 2%) needs to be accepted as “normal” by the current beef industry. *Burdizzo* castration of pre-pubertal (8 months of age) Holstein bulls has also been studied as an animal management practice, in order to improve carcass and meat quality. For instance, results from this study stated that castration improves the grade of backfat classification, the intramuscular fat content, colorimetric parameters, and tenderness. Furthermore, as the optimal tenderness might be achieved in castrated animals without a long ageing period, it represents a good competitive advantage for the Spanish beef industry. However, *Burdizzo* castration might fail in 23% of the cases, and might reduce the total weight gain during the finishing phase. Additionally, the practice of castration illustrates the delicate balance between animal welfare and some management practices on the farm. Therefore, further research (specially in acute and chronic pain and stress) will be necessary to ensure that castration is a good method of improving the meat quality in Holstein bulls. Finally, the supplementation of specific omega-3 rich-ingredients in the ruminant diets has also been proposed as a strategy to increase the carcass and meat quality. Effectively, the supplementation of whole linseed of up to 12% of total dry matter intake, enhances meat quality, and additionally converts meat into functional food (meat rich in omega-3), without affecting animal performance and rumen fermentation. The opportunities for expansion of the market seem to be quite favourable and the interest of the consumers is quite high, but the diffusion of these products in the community area is slowed down by some obstacles, including certification, the prices of whole linseed, and its availability.

The second strategy proposed to increase the efficiency of intensive beef production has been the reduction of feeding cost through the use of industrial by-products. Indeed, the study of the effect of the inclusion of crude glycerin up to 12.1% of total dry matter intake, as an alternative energy source, to substitute cereals in the diet, has been proposed. Fortunately, the inclusion of crude glycerin of up to 12.1%, does not incur any negative effects in performance, ruminal

fermentation, metabolism, animal health, or carcass and meat quality parameters. However, today the reduction of feeding cost through the inclusion of crude glycerin may not be a feasible strategy as a result of the high price of crude glycerin in relation to other concentrate ingredients.

In summary, the present thesis not only provides the results of different feeding and management strategies to improve intensive beef production efficiency, but also highlights concerns about their constrains and limitations.

RESUM

En la present tesis s'han estudiat estratègies per incrementar l'eficiència de la producció intensiva de vedells mitjançant pràctiques de maneig i d'alimentació.

La primera estratègia plantejada per augmentar l'eficiència de la producció intensiva de vedells ha estat la millora de la qualitat de la canal i la carn. Per aquesta raó, es va desenvolupar un estudi per avaluar els efectes de diferents factors pre-sacrifici sobre la incidència de carns amb pH alt o canals amb danys tissulars extrems, i per aconseguir propostes i decisions tècniques de maneig per disminuir la incidència de carns amb pH alt o canals amb danys tissulars extrems. Desafortunadament, l'objectiu *per se* no es va assolir perquè la variablititat del pH últim de la carn i la incidència de canals amb dany tissular extrem explicada per aquests factors va ser molt baixa. Conseqüentment, la indústria càrnia ha d'acceptar com a "normal" un percentatge de canals amb pH elevat (per sobre 13%) i/o presència de dany tissular extrem (per sobre 2%). La castració pre-pubertal de vedells Holstein (8 mesos d'edat) mitjançant *Burdizzo* també s'ha estudiat com a pràctica de maneig per millorar la qualitat de la canal i la carn. De fet, els resultats d'aquest estudi demostren que la castració millora la classificació d'engreixament de la canal, el contingut de greix intramuscular, el valor dels paràmetres colorimètrics, i la tendresa. A més a més, l'assoliment d'una tendresa òptima en els animals castrats sense pràcticament temps de maduració, representa una avantatge competitiva en la indústria espanyola de la carn. No obstant, la castració mitjançant el mètode *Burdizzo* pot fallar en un 23% dels casos, i reduir el guany de pes total durant la fase d'acabat. A més a més, la castració il·lustra la delicada situació relacionada amb temes de benestar animal i pràctiques de maneig a la granja, per tant, més investigació relacionada amb els efectes de la castració sobre el dolor crònic i agut i l'estrès és necessària per assegurar que és una bona estratègia per augmentar la qualitat de la carn i la canal de vedells Holstein. Finalment, la suplementació en les dietes dels vedells amb ingredients rics en omega-3 també s'ha proposat com a estratègia per augmentar la qualitat de la canal i la carn. Efectivament, la suplementació a les dietes amb llavor de lli per sobre el 12% en el total de matèria seca ingerida, augmenta la qualitat de la carn, i a més converteix la carn en un producte funcional (carn enriquida amb omega-3), sense afectar la producció animal i la fermentació ruminal. La oportunitat d'expansió en el mercat sembla favorable i l'interès dels consumidors és elevat, però la difusió d'aquest productes necessita de la superació de la legislació per a la certificació, així com el preu elevat de les llavors de lli i la seva disponibilitat.

La segona alternativa plantejada per augmentar l'eficiència de la producció intensiva de vedells ha estat la reducció dels costos d'alimentació a través de la utilització de subproductes de la indústria. Per aquesta raó, s'ha proposat estudiar els efectes de l'inclusió de glicerina per sobre el 12% en el total de matèria seca ingerida, com a ingredient energètic alternatiu als cereals. Amb

èxit, la inclusió de glicerina com a ingredient energètic no ha afectat negativament els índexs de producció animal, la fermentació ruminal, el metabolisme, i els paràmetres de qualitat de la canal i la carn. No obstant, avui en dia, la reducció dels costos d'alimentació a través de la inclusió de glicerina pot no ser una bona estratègia degut al seu alt cost en relació als altres ingredients.

En resum, la present tesis no només ha proporcionat resultats sobre diferents estratègies de maneig i alimentació que milloren la eficiència de producció intensiva, sinó també informació sobre les seves limitacions i inconvenients.

LIST OF ABBREVIATIONS

a*	Redness colour
ADF	Acid Detergent Fiber
ADG	Average Daily Gain
ADP	Adenosine Diphosphate
ALA	α-Linoleic Acid
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemist
ATP	Adenosine Triphosphate
b*	Yellowness colour
BAND	Castration through rubber rings or latex bands
BSE	Bovine Spongiform Encephalopathy
BURD	*Burdizzo* castration
BW	Body Weight
CAP	Common Agricultural Policy
CCW	Cold Carcass Weight
CLA	Conjugated Linoleic acid
cm	Centimeters
CP	Crude Protein
d	Day
DHA	Docosahexaenoic Acid
DM	Dry Matter
DMI	Daily Matter Intake
EC	European Commission
EE	Ether Extract
EPA	Eicosapentaenoic Acid
FA	Fatty Acid
FAME	Fatty Acid Methyl Ester
FAO	Food and Agriculture Organization
g	Gram
GC	Gas Chromatography
GLC	Gas-Liquid Chromatography
G : F	Gain to Feed ratio
GH	Growth Hormone
kg	Kilogram
h	Hour
HCW	Hot Carcass Weight
IGF-I	Insulin-like Growth Factor I

im	intramuscular
IRR	Incidence Relative Ratio
IRTA	Institut de Recerca i Tecnologia Agroalimentàries
IU	International Unit
L	Liter
L*	Lightness
LA	Linoleic Acid
LL	Lipid Level
LM	*Longissimus* muscle
LS	Lipid Source
m	Meter
ME	Metabolizable Energy
min	Minute
mg	Miligram
mL	Mililitre
mM	Milimolar
mmol	Milimol
ND	Non detectable levels
NDF	Neutral Detergent Fiber
NEFA	Non-Esterified Fatty Acid
NFC	Non-Fiber Carbohydrates
NIT	Near Infrared Transmission
NRC	National Research Council
ng	Nanogram
n-3	Omega-3
n-6	Omega-6
OM	Organic Matter
OR	Odds Ratio
PUFA	Polyunsaturated Fatty Acid
SD	Standard Deviation
SEM	Standard Error of the Mean
SFA	Saturated Fatty Acid
SUR	Surgical castration
VFA	Volatile Fatty Acid
WBSF	Warner-Bratzler Shear Force
WHO	World Health Organization
wk	Week
wt	Weight

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CHAPTER I: INTRODUCTION

CHAPTER I

1. INTRODUCTION

In the second half of the last century, beef production priorities *per se* have been centred on productivity and, most recently, in efficiency (Euclides, 2004). This efficiency takes into account the magnitude of input of production relative to output (net-profit) or, equivalently, quantity of carcass and meat adjusted for its quality (Dickerson, 1970). Although feed is a major cost component in the animal production system, accounting for approximately half of variable costs (Herd, 2003), the efficiency of intensive beef production also needs to consider the calf purchase price (Departament d'Agricultura, Alimentació i Acció Rural, 2008), the growing concerns about sustainable development (Meadows et al., 1992), animal welfare standards and good practices (LMC Bulletin, 2007), as well as food safety (Sofos, 2008).

Efforts to improve the efficiency of intensive beef production in Spain have mainly focused on reducing animal feeding costs by using alternative feed ingredients, and/or to increase the feed efficiency through both genetic and non-genetic means. However, few efforts have been carried out to improve the intensive beef production efficiency through increasing the quantity, and quality of carcasses, and meat during the finishing period. Although beef quality has become a complex and dynamic issue, involving the total chain from fork-to-farm with a multitude of interacting aspects related to producers, slaughtering companies, and wholesalers to retailers, and consumers, some of the best strategies to enhance carcass and meat quality are modifying animal management practices and/or feeding animals with different dietary sources.

2. DESCRIPTION OF INTENSIVE BEEF PRODUCTION

2.1. INTRODUCTION

Beef is an important part of the diet in the European Union (EU). More than half of the beef for consumption in the EU is sold as fresh beef products at the retail level, the rest being used in processed products (Nielsen, 2001). The EU-25 is the world's third largest producer of beef (13%) after the USA (24%), and South America (21%; mainly Argentina and Brazil, "Mercosur"). The EU as a whole is self-sufficient in beef, and since the beginning of the nineties has been one of the world largest exporters (Nielsen, 2001). In fact, the EU is the fourth world exporter after Australia, the USA, and Brazil (Chatellier et al., 2003). At the same time, large differences in production exist across individual countries. France, Germany and Italy account for over 56% of total production, while Spain represents 8.5% of total EU production (Libro Blanco, 2003), making beef production an important socioeconomic factor. However, from 2005 to 2006 beef production in the Spain went down (-5.92%) from 2,445,295 to 2,757,558 million animals slaughtered in 2005 and 2006, respectively (Ministerio de Medio Ambiente y Medio Rural y Marino, 2008), and the average *per capita* of beef consumption was reduced from 20 kg to around 10 kg/head/year (Ministerio de Medio Ambiente y Medio Rural y Marino, 2007).

In Spain, where the predominant climatic conditions difficult forage production (quality and quantity), beef production is manly based on intensive systems (Bacha et al., 2002), although some local and rustic (non-improved type) beef breeds are reared under traditional systems (Serra et al., 2004). The intensive production system is completely detached from the land, and calves (early-maturing breeds) are separated from their mothers at 1 to 2 days of age, and artificially reared on milk or milk replacer plus solid food for a 6 to 12 week period. After that, animals are fed *ad libitum*, with concentrate and straw, until achieving the target slaughter weight of 400-460 kg (Departament d'Agricultura, Alimentació i Acció Rural de Catalunya, 2008). Concentrates are formulated with high concentrations of crude protein and energy (crude protein from 13.8 to 18%; energy from 2.50 to 2.90 ME/kg on a DM basis). The most common feed ingredients used in diet formulation are products and by-products of cereals (such as distillers grains, corn gluten feed, wheat meddlings), and soybean. Little human intervention to fed animals is required because silos are filled with concentrate, usually once each 7-14 several days. The animals in this system attain an average daily gain (ADG) between 1.2 and 1.4 kg per day, from a dry matter intake (DMI) of from 5.3 to 9.8 kg per day, and a gain to feed ratio (G: F) from 0.18 to 0.25 (Table 1), although there are considerable variations among age, breed, and nutrition schedule (Albertí et al., 1997; Bacha et al., 2002; Piedrafita et al., 2003; Bacha et al., 2005).

Table 1. Performance data of young bulls feed high-concentrate diets (Ferret et al., 2008, in press)

Animal	Initial and final BW (kg)	Fattening period (Days)	ADG (Kg/d)	Average concentrate intake (kg/d of DM)
Holstein Male	50-480	346	1.32	6.22
Holstein Female	51-375	337	1.10	6.00
Crossbred Male	65-495	356	1.50	6.00
Crossbred Female	70-390	334	1.20	5.50

2.2. EFFICIENCY OF INTENSIVE BEEF PRODUCTION

It is not feasible to measure the efficiency of intensive beef production, as this would require measurements of multiple input and output. While the main factors that influence the efficiency of any beef production system include calf purchase price, and feed ingredients price, the foremost output that influences efficiency is the final carcass weight, its characteristics, and its market price. Whereas from 2001 to 2006 intensive beef production has been reporting benefits, due to steadily increasing beef prices, low cereal cost, and beef premia payments, recently it is difficult due to the higher cereal prices and higher rates of inflation. In addition, the beef premia payment has been reduced since the reform of the Common Agricultural Policy (CAP) adopted by EU farm ministers in 2003. Nowadays, producers received approximately 26.40 € per each slaughtered animal older than 8 months (Ministerio de Medio Ambiente y Medio Rural y Marino, 2008), whereas in 2002 producers received 126 € per each fattened animal plus 80 € per each slaughtered animal older than 8 months (Ministerio de Medio Ambiente y Medio Rural y Marino, 2008). The way the EU supports the agricultural sector has changed, confronting producers with an increasing number of regulations and more administration (Oenema, 2004). The regulations are rather complex, and depend on allocation, intensity, size of production, as well as the respect for the environment, food safety, and animal welfare standards (Directorate General for Agriculture, 2002). More money is available to farms that respect environmental preservation (premium related with extensification), and quality of animal welfare. In contrast, direct payments for bigger farms are reduced, as a result of current perception that intensive beef production has various negative effects on the environment (Oenema, 2004).

2.3. STRATEGIES TO INCREASE THE OUTPUT OF INTENSIVE BEEF PRODUCTION

2.3.1. Introduction

Although a considerable amount of effort has been made to improve beef production efficiency, few studies have taken into account improvements in terms of quality and quantity of beef carcass and meat, with due consideration given to consumer demand-perception, economic, environment and animal welfare consequences. Although beef carcass and meat quality is a complex and dynamic issue, involving the total chain from farm to fork with a multitude of interacting aspects related to producers, slaughtering companies, and wholesalers to retailers, and consumers, it is important to improve carcass and also meat quality characteristics, leading to products with an added value, and to face the large and competitive meat industries of Argentina, Brazil and USA (Van Trijp and Steenkamp, 2005; Gellynck et al., 2006).

Meat quality characteristics are classified into sensorial (associated with colour, flavour, juiciness), nutritional (associated with crude protein content, fat content, amino acids profile, fatty acids profile, vitamins), food safety (associated with microbial pathogens like *Escherichia coli* O157: H7 and *Listeria monocytogenes,* and food additives, chemical residues, and products of food biotechnology or genetically modified organisms), and technological categories (associated with tenderness, water holding capacity, pH, moisture). In general, the meat quality information reaches the consumer in the form of quality cues, which are defined by Steenkamp (1997) as informational stimuli that, according to the consumer, say something about the product. Cues can be intrinsic and extrinsic. Intrinsic cues are related to physical aspects of the meat (cut, colour, intramuscular fat content, tenderness, flavour, juiciness), whereas extrinsic cues are related to non-physical aspects of the product (price, origin, stamp of quality, production system and nutritional information). These cues are categorised and integrated for the consumer to infer the quality attributes of meat (Bernués et al., 2003). Additionally in Spain carcass quality characteristics are mainly defined by the backfat (fat cover), and conformation, according to the EU classification system into 1.2.3.4.5 (EU Regulation nº 1208/81) and into (S)EUROP categories (EU Regulation nº 1208/81, 1026/91), respectively. Also hot carcass weight, ultimate meat pH, and extreme bruises are used to specify the value-based marketing of carcass (Sañudo and Campo, 1997). These value-based marketing of carcass involves: 1) payment of incentive to producers capable of supplying animals that meet specific market requirements, and 2) high discounts (around 20 to 30%) when carcass presents bruises, has lower conformation and backfat, or has a high ultimate pH.

Until the present, feeding strategies have been the management factor most actively studied to increase quality of carcass and meat during the finishing phase, leading to products with an added value in this more saturated food market. The latter includes uptake and incorporation of specific feeding components that contribute to lipid content and composition in relation to nutritional value (Wood and Enser, 1997), or influences on technological quality and storage life (Sheard et al., 2004). Additionally genetic breed selection (Keane, 1994; Serra et al., 2004; 2008), gender type selection (Sueiro et al., 1994; Fiems et al., 2003), modifying the age of slaughter (Shackelford et al., 1994) or final body weight (Colomer-Rocher et al., 1980; Sánchez et al., 1997), management production practices (Knight et al., 1999a; 1999b; Realini et al., 2004), and pre-slaughter management (Warriss, 1990) factors have been studied to increase carcass and meat quality. Furthermore, it is possible to increase meat quality through selection methods for raw meat, processing technologies, packaging and distribution systems, delivering “easy to handle”, “ready to cook” or “ready to eat”. At the processing stage, beef meat quality (specially, nutritional quality) can also be increased by reducing the levels of food ingredients and additives with proven negative impacts on human health, and by adding health promoting ingredients, such as micronutrients, probiotics, and other functional food ingredients. Each link in the distribution chain from farm through slaughterhouse to retailer is important in order to improve the carcass and meat quality.

2.3.2. Enhancing carcass and meat quality by modifying the animal management practices

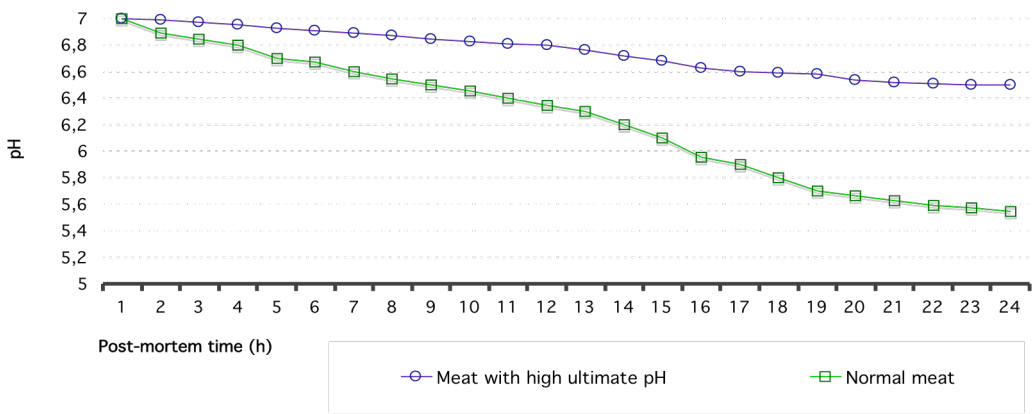
Recently, the Spanish beef industry has reported an increase in the incidence of meat with high ultimate pH. Changes in the pH during the *post-mortem* period negatively influence the intrinsic characteristics of the meat (Mounier et al., 2006), especially qualities most appreciated by the consumers, e.g. tenderness, juiciness and flavour. *De facto*, meat with high ultimate pH presents a dark red colour (Bartos et al., 1993), greater water holding capacity (Apple et al., 2005), and poor palatability (Viljoen et al., 2002). From a microbiological point of view, the meat with high ultimate pH has a more rapid growth of microorganisms to unacceptable levels with the development of off-odours, and often slime formation (Pipek et al., 2003; Mach et al., 2007). These changes in the pH reduce the value of the carcasses, representing a serious economic problem in the meat industry. Frequently, the discounts associated with meat ultimate pH greater than 6.0 are around 150 € per animal (Data not published, IRTA 2008).

After exsanguination, as soon as blood flow ceases, muscle cells are subjected to hypoxia and soon after to hyperosmotic conditions. The cell responses to these stresses are multiple. With the development of hypoxic conditions after bleeding, the first modification is very likely to be a rapid decline of the pH as a result of lactic acid produced from anaerobic glycolysis (Figure 1). This feature is probably the major cause of the amplitude of the pH decline always observed upon the 30 to 60 min *post-mortem*. In fact, it is stated that the causes of muscle acidification are multiple. When phosphocreatine stores are exhausted, the required energy is mainly produced through the

anaerobic degradation of glycogen by glycolysis.

Glycogen is a branched polysaccharide of α-D-Glucose unit. In beef, muscle glycogen concentration ranges between 75 and 120 μmol/g (Immonen et al., 2000), and depends on the net result of utilization and production by muscle (Immonen et al., 2000). The average rate of glycogen break down was reported to be 10 to 11 μmol/g/h (range 5 to 24) in young bulls severely stressed by co-mingling or adrenaline administration (Tarrant and Lacourt, 1984), whereas the rate of repletion is slow, 1.6 μmol/g/h (McVeigh and Tarrant, 1983). Not all muscle glycogen is synthesized from glucose originating directly from liver glycogen; some glucose absorbed from the alimentary tract may serve directly in muscle glycogen synthesis.

Figure 1. A schematic representation of the rate of pH decline *post-mortem* in normal beef meat and in beef meat with high ultimate pH (Warriss, 1990)



About 45 μmol of glycogen is needed to lower the pH of 1 kg of muscle from 7.2 to 5.5 (Immonen et al., 2000). This equals to 7g/kg of muscle. Warriss (1990) presented a figure on the relationship of ultimate pH to the concentration of glycogen present in the LM at death. Data on the curvilinear dependence consisted of 2,345 observations and revealed that pH decline appears to be limited only below glycogen concentration of 45 μmol/g. Various authors have reported glycogen concentration at the time of slaughter and the corresponding ultimate pH values. Lahucky et al. (1998) measured glycogen concentration immediately prior to slaughter from control and stressed bulls. The glycogen concentrations were 61 and 33 μmol/g, producing pH values of 5.66 and 6.70, respectively. Immonen and Poulanne (2000) found that at pH values below 5.75, bovine muscle residual glycogen concentrations varied from 10 to above 80 μmol/g, showing that pH is a relatively “insensitive” measure for understanding the physiology of muscle (e.g. after physical and physiological stress), since the muscle glycogen in the live animals must be depleted about 70 to 80 μmol/g without changes in pH.

Presumably, *post-mortem* glycolysis and muscle pH decline is stopped, under normal carcass chilling conditions, when muscle pH declines to approximately 5.45, and this low pH inhibits the activity of glycolytic enzymes, or when muscle glycogen concentrations are depleted. However, the rate of the pH decline also depends on the efficiency of the glycolytic pathway, and the buffering capacities of muscle cells. The very fast decline of pH immediately after bleeding can probably not only be ascribed to glycolysis and the subsequent accumulation of lactic acid. This was supported by the increase in the level of enzymes involved in the oxidative and the glycolytic pathways within 20 min after slaughter, two pathways providing the energy needed to preserve cells from death and/or setup the program cell death machinery (Jia et al., 2006ab). The findings of Jia et al. (2006ab) further demonstrate that protein synthesis takes place after animal bleeding and prove the intense metabolic activity of *post-mortem* muscle cells suggested before. Replacement of acidic components (phosphatidylserine) by basic components (phosphatidylcholine and phosphatidylethanolamine) in the intracellular compartment, accompanied by a redistribution of ions, could explain the existence of transient pH stability steps (Ouali et al., 2006), which occurs between 1 and 8 h *post-mortem*. Additionally, glycogen concentration shows an inherently variable nature dependent on breed, nutritional status of animal, physical exhaustion and psychological pre-slaughter stress, electrical stimulation, and the type of muscle (e. g. oxidative vs. glycolytic). Each muscle fiber type has different biochemical and biophysical characteristics such as oxidative and glycolytic capacities, contraction speed, fiber size, myoglobin, and glycogen concentration (Brandsteteer et al., 1998). Muscle fiber type I has slow-twitch, oxidative metabolic characteristics, and a low glycogen concentration, whereas type II A is a fast oxidative–glycolytic fiber. On the other hand, type IIB has fast-twitch, glycolytic metabolic characteristics, and a high glycogen concentration (Karlsson et al., 1999). Thus, muscles with different fiber type composition have different effects on *post-mortem* glycogen concentration, and may have a subsequent influence on ultimate meat pH (Ozawa et al., 2000).

As described below, physical exhaustion and psychological pre-slaughter stress of cattle might reduce the glycogen concentration (Immonen and Puolanne, 2000). During pre-slaughter phase animals can be exposed to a range of challenging stimuli including: time, handling and increased human contact, loading and unloading, novel/unfamiliar environments, food and water deprivation, changes in social structure (e. g. through separation and mixing animals from different farms and/or pens), high stocking densities during transportation or at the slaughterhouse, and changes in climatic conditions. In fact, this physical exhaustion and psychological pre-slaughter stress perturb the animal well being (fear, dehydration, fatigue, physical injury), and an adaptive response is activated in an attempt of restore balance (Ferguson and Warner, 2008). Adaptive response can be non-specific, and considerable variability exists between animals not only in their perception of the stressor but also in their coordination of the response (Moberg, 2001). Several intrinsic animal factors (e. g. genetics, sex, age, and physiological state), past experiences, and acquired learning (Moberg, 2001), the type, duration, and intensity of individual pre-slaughter

stressors might affect the adaptive response (Ferguson et al., 2001). The activation and regulation of the neuroendocrinal response to fear-eliciting stimuli has been studied extensively by Steckler (2005). The two central integrated processes include the autonomic nervous system and hypothalamic-pituitary-adrenal (HPA) axis. It has been reported that the secretion of catecholamine affects the incidence of high ultimate meat pH as a result of significant changes in energy metabolism including lipolysis, glycogenolysis and gluconeogenesis (Kuchel, 1991).

In fact, the high incidence of ultimate meat pH might be mitigated by the repletion of muscle glycogen concentration. In humans, the rate of muscle glycogen recovery is optimized by consumption of 1.0 g of carbohydrate supplement per kilogram of body weight immediately after cessation of exercise (Ivy et al. 1988), although full recovery within 24 h requires a total intake of 500 g of carbohydrate (Costill et al., 1981). Adding small amounts of protein (0.3 to 0.34 g/kg of body weight) increased the rate of muscle glycogen storage in humans due to the synergistic insulin response produced by the combination of protein and insulin (Zawadzki et al., 1992). In lambs, Chrystall et al. (1981) reported that muscle glycogen slowly replenished after transport and exercise despite denial of food and water. Gardner et al. (2001), feeding cattle with 4 dietary treatments: hay, silage, hay-barley, and hay-maize reported that after the exercise regimen (cattle were trotted at 9 km/h for five 15-min intervals), glycogen concentration repleted in a linear fashion over 72 h in the M. *semimenbranosus* of the animals fed maize, barley, and silage. In contrast, the M. *semitendinosus* of these animals was refractory to glycogen repletion over the same period. Both the M. *semimenbranosus* and M. *semitendinosus* of the cattle on the hay diet showed no significant repletion following exercise, suggesting a positive linear relationship between glycogen repletion and ME intake.

Great attempts have been made to increase the availability of glucose to ruminants before slaughter, and thus, enhance muscle glycogen synthesis and storage, although results have not always been achieved. Propionate produced by the ruminal fermentation is the main precursor for hepatic glucose production in the ruminant. Therefore, several studies have focused in feeding diets rich in highly fermentable carbohydrate prior to pre-slaughter stress, in order to increase the amount of propionate acid. Additionally, the administration of selective antibiotics, such as monensin, to reduce methane energy losses and to promote increased propionate production, or the use of direct-fed microbial products has been studied. Feeding nutrients in a form that will largely protect them from fermentation without affecting enzymatic degradation lower down the gastrointestinal tract, have also been investigated with the objective to increase the availability of glucose to ruminants (Leek, 1993). Gardner and Pethick (2005) reported that glycerol and propylene glycol mixed in drinking water at the rate of 3.5% and 1.5%, respectively, during 24 h of waiting time at slaughterhouse, were effective in reducing the ultimate pH of cattle about 0.1-pH unit. In addition, Parker et al. (2007) reported that steers orally dosed at 24 and 48 h before slaughter with glycerol (2 g/kg BW) presented greater glucose concentrations that non-

supplemented steers, and suggested that elevated blood glucose concentration in the glycerol treated animals may provide a preferential fuel for liver gluconeogenesis. Additionally, although full-feeding cattle on arrival at packing plants is not practical under commercial conditions, Schaefer et al. (1999) suggested that a low rate of supplementation of a concentrate diet would have beneficial effects on tissue catabolism of stressed animals. In fact, Schaefer et al. (1999) stated that modification of the diet for a short period (1 to 2 d) immediately before cattle transport and (or) providing a relatively small amount of a nutrition supplement during waiting time at the slaughterhouse (e.g. ions, and amino acids) offers the potential to improve carcass yield, and reduce meat quality defects.

Additionally, great attempts have been made to decrease the depletion of glucose in ruminants before slaughter through management practices. In fact, the effects of stress factors on muscle glycogen depletion and the consequent incidence of high ultimate meat pH have been well documented (Ferguson et al., 2008). However, there has been little examination of the consequence of the interaction of those pre-slaughter factors (concerned with animal, farm, transport and slaughterhouse) on meat ultimate pH.

In addition to the important incidence of meat with high ultimate pH, the Spanish market today is facing other carcass and meat quality problems. The optimal hot carcass weight (HCW) is between 272 and 340 kg. In addition, the optimal carcass conformation is the “R” category (when profiles on the whole are straight with good muscle development), and the optimal degree of carcass backfat is the “3” category (when carcasses present flesh, with the exception of the round and shoulder, and are covered with fat, with slight deposits of fat in the thoracic cavity). However, in Holstein bulls (70% of total bulls produced in Catalonia), 86.9% of their carcasses are classified as “O” in conformation, and 44.6% of carcasses are classified as “2” in backfat following EU Regulation (Data from Mercabarna Slaughterhouse, 2007). Carcasses from Holstein young bulls clearly present less conformation and backfat than that desired in the Spanish market. For instance, for young Holstein bulls with hot carcass weights < 300 kg, the economic losses between “R” or “O” conformation category are close to 37 € per animal (Mercabarna, 2008). One way to solve these carcass and meat quality problems with Holstein young-bulls could be castration. Castration of bulls increases intramuscular fat content (Knight et al., 1999ab), carcass backfat (Field, 1971; Knight et al., 1999a), which determines carcass final prices, and tenderness (Morgan et al., 1993). The differences between carcass and meat quality of castrated animals and intact bulls are mainly associated with an inhibition of anabolic hormones produced by the testes (Adams et al., 1996). Therefore, Boccard et al. (1979) and Mc Cormick (1992) reported that castrated animals presented lower concentrations of hydroxyproline (the main component of collagen protein) than intact bulls, as a result of the lack of the anabolic effects of testosterone on collagen synthesis. Although these studies have linked bull meat tenderness to greater amounts of connective tissue, the proteolysis may be affected by castration. Morgan et al. (1993) reported an

important relationship between enzymes activity at 24 h *post-mortem*, myofibrillar proteolysis, and meat tenderization in castrated animals. Morgan et al. (1993) reported greater amounts of proteolysis in muscle from castrated animals during the first 7 d *post-mortem* than in intact bulls, probably as a result of the greater μ-calpain activity. Probably, proteomic techniques will be a good instrument to enhance the relatively little knowledge of the changes in the total tissue proteome after castration, and during ageing.

On the other hand, as a result of the changes in complex system of growth factors like growth hormone (GH), insulin-like growth factor I (IGF-I), insulin, thyroid hormones and anabolic hormones produced by the testes, castrated animals exhibit lower growth rates and feed efficiencies, and dressing percentages than intact males. Anabolic hormones produced by the testes are responsible for the differential in growth between intact males and castrated animals, as testosterone is a potent muscle growth stimulant that counteracts fat deposition. In addition, while castration can contribute to improvements in beef carcass quality, attention must be given to animal welfare. The LayWel report (Bessei, 2005) reported that most definitions of welfare include physical, physiologic, and psychological/mental aspects. Indicators of poor welfare (decrease growth, body damage and illness, and increase in abnormal behaviour) can be used to assess animal welfare after castration management. Therefore, in order to optimize the animal welfare related to castration, further research (specially in acute and chronic pain and stress) is needed to ensure that castration is a good method to improve the outputs of intensive beef production system.

2.3.3. Enhancing meat quality by feeding animals from different dietary sources

In the food guide pyramid, meat is categorized as a protein food group along with poultry, fish and eggs. Undoubtedly, meat is a major source of food proteins with a high biological value in many countries (Arihara, 2006). Meat is also an excellent source of some valuable nutrients such as minerals and vitamins (Biesalski, 2005). Regrettably, over the last 10-15 years, these positive attributes have often been overshadowed due to the perception that beef contains high amounts of fat, which has been related to some diseases when consumed in excess. Today, it is accepted that both, the amount and the profile of the fatty acids, are risk factors in the development of some diseases. Therefore, it is recommended that people should decrease their intake of saturated fatty acids (less than 10% of the total calories) and *trans*-fatty acids (less than 1%), increase the intake of unsaturated fatty acids (more than 0.5%), and decrease the omega-6 (n-6) to omega-3 (n-3) ratio fatty acids in the diet to levels 5 to 1 (World Health Organisation (WHO), 2003). More recently, Wijendran and Hayes (2004) have described the importance of providing a ratio of n-6 to n-3 fatty acid close to 6.0 in human diets, but have also emphasized, when contemplating long-term consumptions of fatty acids, that the first consideration should be the absolute amounts of n-6 and n-3 consumed, rather than their ratio.

In that sense, Wijendran and Hayes (2004) recommended 1.7 g/d of α-linolenic acid (ALA) based on the reduction of platelet aggregation in hyperlipidemic subjects when they consumed this ALA amounts daily (Freese et al. 1994).

As consumers are increasingly aware of the relationships between diet and health, particularly in relation to cancer, atherosclerosis and obesity/type 2 diabetes, efforts have been made by the food industry to convert products into a functional food. Functional food is a food similar in appearance to a conventional food, consumed as a part of the usual diet, which contains biologically active components with demonstrated physiological benefits (Food and Agriculture Organization (FAO), 2004). Examples of benefits of functional foods are anticarcinogenicity, antimutagenicity, and antioxidative activity (Arihara, 2006).

The most intensively investigated functional foods are those enriched with n-3 fatty acids (Hasler, 2002). The n-3 fatty acids are predominantly found in fatty fish such as salmon, tuna, sardines and herring (Kris-Etherton et al., 2000; Lee and Lip, 2003). The n-3 fatty acids, especially α-linolenic acid (ALA, *cis*-9, *cis*-12, *cis*-15-18: 3), eicosapentaenoic acid (EPA, *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17-20: 5), and docosahexaenoic acid (DHA, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19-22: 6) have been reported to exert beneficial effects during growth and development, and prevent and treat cardiovascular diseases, inflammatory and autoimmune disorders, cancer, depression, and psychological stress (Hasler, 2002; Lee and Lip, 2003; Larsson et al., 2004; Logan, 2004).

It is known that intake of n-3 fatty acids is much lower today than at the beginning of the last century due to decrease in fish consumption, and the increase in meat consumption from animals fed with concentrates rich in grains containing n-6 (Mandell et al., 1997; Sanders, 2000). The ratio of n-6 to n-3 fatty acids can be improved by decreasing n-6 consumption, increasing n-3 consumption, or both. As a result of this lack in n-3 fatty acids consumption, there has been a great deal of interest in enriching beef with n-3 fatty acids by modifying the ruminant diets to respond to consumers demands (Scollan et al., 2001, 2003). The main sources of supplementary n-3 fatty acids in ruminant rations are plant oils and oilseeds (mainly linseed oil and whole seed), fish oil, marine algae, and fat supplements (Givens et al., 2000). Fish oil is rich in n-3 fatty acids, specially the long-chain n-3 fatty acids EPA and DHA, but it is not well accepted by the producers, also the concentrations of EPA and DHA are dependent of the species of fish and represents, at most, 25% of fish oil fatty acids, the rest often being rich in saturated fatty acids (Givens et al., 2000). A prudent strategy would be to concentrate these fatty acids prior to ruminal protection. On the other hand, marine algae are not included in ruminant diets because of their high price. Feeding oilseeds to beef is one of the best methods of enhancing the proportion of n-3 fatty acids in meat. Linseed would be a good choice from the consumer point of view, being a source of linolenic acid (56% of the total fatty acids). To enrich beef with n-3 fatty acids, the dietary supply of n-3 fatty acids must escape rumen biohydrogenation (which converts unsaturated fatty acids to saturated fatty acids)

before it can be absorbed in the small intestine and deposited in meat. One strategy to avoid rumen biohydrogenation is to feed whole oilseeds, because the seed coat prevents the access of rumen microorganisms to the unsaturated fatty acids (Aldrich et al., 1997). Additionally, a variety of procedures have been explored including the use of heat/chemical treatment of whole/processed oilseeds, rolled or cracked whole oilseeds, chemical treatments of oils to form calcium soups or amides, emulsification/encapsulation of oils with protein and subsequent chemical protection, in order to increase the amount of n-3 fatty acids in tissue (Ashes et al., 2000). Hence, for example, using the later technology, Scollan et al. (2003) showed that a protected plant oil supplement markedly improved the polyunsaturated to saturate ratio (from 0.08 to 0.27) by increased the n-6 to n-3 fatty acids ratio (from 2.75 to 3.59) in beef muscle. However, Choi et al. (2000) and Raes et al. (2004) also increased the n-3 fatty acid content of muscle in late maturing breeds of cattle by feeding forage-based diet supplemented with oils or extruded and crushed linseed rich in ALA, EPA or DHA. It is noteworthy that feeding fresh grass or grass silage compared to concentrates, rich in n-3 and n-6 fatty acids, respectively, also results in greater concentrations of n-3 fatty acids in muscle lipids, both in the triacylglycerol and phospholipids fractions (Nuernberg et al., 2005). Significantly, grass compared to concentrate feeding not only increased n-3 fatty acids muscle phospholipids but also EPA, DPA and DHA (Dannenberger et al., 2004). Studies in Ireland showed that both the proportion of grass in the diet and length of time on grass were important in determining the response in beef fatty acids (Noci et al., 2005).

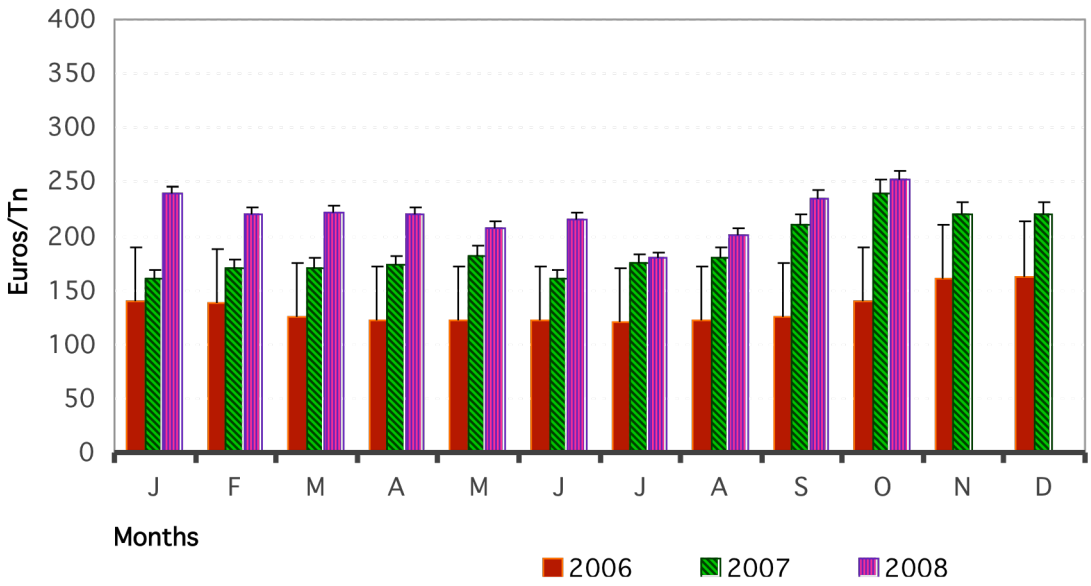
However, in Europe the demand for functional foods varies remarkably from country to country, on the basis of the alimentary traditions, the enforced legislation, and the different cultural heritage that people have acquired. The opportunities of expansion on the market seem to be quite favourable and the interest of the consumers is rather high, but the diffusion of these products in Spain is slowed down by various obstacles, including certification legislation and prices of purchase ingredients (specially oilseeds).

2.4. INPUT OF INTENSIVE BEEF PRODUCTION

The main cost of intensive beef production is the feed cost during the growing and finishing phase (Herd et al., 2004), followed by the cost of buying calves (Departament d'Agricultura, Alimentació i Acció Rural, 2008). Properly managing of feed ingredients and calf purchase is critical to the success of the beef production efficiency. Nowadays, in Catalonia the purchase price of Holstein calves aged between 1 and 3 week averages 170 euros/animal, although in February 2008 it had been reduced by 13.37% compared with August 2006 (Ministerio de Medio Ambiente y Medio Rural y Marino, 2008). This reduction of calf purchase price was similar in France (104 euros/animal in November 2007, 32% less compared with November 2006), Ireland and Germany (82 and 89 euros/animal in November 2007, 14 and 15% less compared with November 2006,

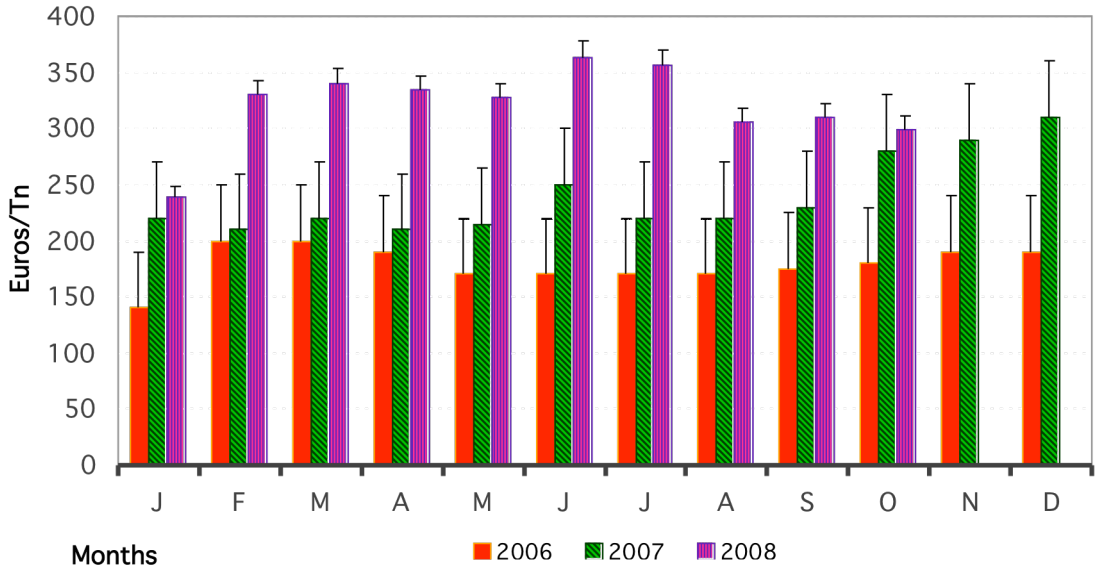
respectively). Today calf purchase prices in Catalonia (and Spain) are greater than in other countries as a result of structural deficit of calves. In fact during 2006, around 550,000 animals were imported, mainly from eastern UE-25 countries (Calcedo, 2008). In contrast, the cost of most commonly feed ingredients used in beef diet formulation has increased above to 40.9% since August 2006 to August 2008 (Departament d'Agricultura, Alimentació i Acció Rural, 2008). This recent situation has made producers to look closely for factors such as feed availability and its prices, feed quality, and alternative feeds for beef. During 2008 springtime and summer season cereal and soybeans prices have risen dramatically (Figure 2 and Figure 3). One of the main contributors in rising cereal and soybean prices is the economic growth in many developing countries, which has led to an increase middle-class consumers, generating an increase in food demand.

Figure 2. Increase in barley price from August 2006 to October 2008 in Catalonia ("Llotja de Lleida"; Departament d'Agricultura, Alimentació i Acció Rural, 2008)



For comparison, in 1990, the middle class grew by 9.7% in India and 8.6% percent in China, as a percentage of their populations, whereas in 2008 it has reached a growth rate of nearly 30% and 70%, respectively (International Monetary Fund, 2008). Another significant contributor that is already pushing up prices and explains, in part, the 40 percent rise in the last year on the food price index calculated by the Organization for Economic Co-operation and Development (2008), is the fact that many grains are being used for ethanol and biodiesel production (Von Braun, 2008), as a consequence of the growing demand for transportation energy, and concerns about global warming.

Figure 3. Increase in soybean price from August 2006 to October 2008 in Catalonia ("Llotja de Barcelona"; Departament d'Agricultura, Alimentació i Acció Rural, 2008)



On the other hand, food prices have also risen as a consequence of the reduction in global stocks of corn, wheat and soybeans, the crop shortfalls from natural disasters, the impact of trade liberalization, and financial speculation (Organization for Economic Co-operation and Development, 2008).

2.5. STRATEGIES TO REDUCE THE INPUTS INTO INTENSIVE BEEF PRODUCTION DURING THE FINISHING PHASE

2.5.1. Introduction

Although in a competitive world market, greater efficiency might be generated through product and market development, innovation, and differentiation, an option for increasing intensive beef production efficiency is to reduce animal feeding cost by using alternative feed ingredients (e.g. by-products), and/or increasing the feed efficiency.

2.5.2. Increasing intensive beef production efficiency by increasing feed efficiency

Feed efficiency is defined as the live weight gain resulting from the daily DMI (Koch, 1963), although output traits can also be expressed as carcass or lean product, and input traits as digestible or metabolizable energy intake (Crews, 2005). Significant improvements in feed

efficiency have been carried out through both genetic and non-genetic means. Avenues for genetic improvement of feed efficiency include choice of breed, crossbreeding, and selection within breeds (Herd et al., 2003), which at the moment are not use to apply to our Holstein beef production system. Non-genetic means the inclusion of feeding strategies and/or additives (Cardozo et al., 2005ab) that will allow producers to maintain the current level of production without increasing the cost. Rumen fermentation enables ruminants to make maximal use of forages that cannot be used to feed pigs or poultry. This fermentation could be improved in many ways, such as by improving fibre digestion, decreasing protein degradation, and/or inhibiting methane emissions, which, if modified, might increase efficiency of beef production, and reduce impacts of animal production on the environment. Ionophores have been used in beef diets because of their ability to improve the efficiency of nutrient utilization by reducing methane energy losses and increasing propionic acid production. In addition, ionophores reduce the risk of ruminal acidosis and bloat (Chalupa et al., 1980). However ionophores in animal feeds have been banned in the European Union since January 2006 (EU 1831/2003). For this reason, the industry is searching for alternative additives such as probiotics (or more accurately in ruminants, direct-fed microbial), and biological feed additives such as enzymes and plant extracts and secondary plant metabolites (Cardozo et al., 2004; Busquet et al., 2005ab), which are generally recognized as safe for human and animal consumption. Calsamiglia et al. (2005) indicated that the combination of additives with different mechanisms of action may result in synergistic effects that may enhance ruminal fermentation.
There is a vast amount of literature available regarding the effect of these feed additives on feed efficiency, as well as their constraints and limitations in the use.

Direct-fed microbial products (DFM) are products that contain live (viable) microorganisms (bacteria and/or yeast). Direct-fed microbial products state, or imply beneficial effects in animals associated with these products content of viable microorganisms (Food and Drug Administration, 2008). There have been some indications that certain bacterial DFM may also have beneficial effects in the rumen. Results have not always been beneficial when animals have been fed DFM. Lack of organism specificity, proper dose, and survival are some reasons for these findings. However, Jeong et al. (1998) fed *Lactobacillus* sp. and *Streptococcus* sp. to lactating cows and reported a 0.8 kg/d improvement in milk production over control cows. Supplementation of *Lactobacillus* may be useful in the close-up dry period of lactation when intake is depressed and animals are stressed. Savoini et al. (2000) reported that cows fed *Lactobacillus* sp. in the transition period produced measurably more milk and had lower blood non-esterified fatty acids, but higher blood glucose than did untreated cows. Additionally, Ghorbani et al. (2002) reported that supplementation of *Propionibacterium* and *Enterococcus faecium* in steers receiving high concentrate diets decrease the risk of acidosis, without inducing changes in dry matter intake or ruminal and blood pH. Additionally, feeding *Saccharomyces cerevisiae* increased the number of rumen protozoa and increased NDF digestion in steers fed straw-based diets (Plata et al., 1994). Also enzymes have been tested as biological feed additives. Enzymes are protein molecules that

catalyze specific chemical reactions. The integrity of the enzyme is not the only criteria that should be studied when evaluating enzymes for ruminant diets, because in order for them to be effective, they must bind to their substrate and catalyze reactions. Enzymes can improve nutrient digestion, utilization, palatability, and productivity in ruminants and at the same time reduce animal fecal material and pollution (Beauchemin and Rode, 1996; Treacher and Hunt, 1997). However, temperature, time, substrate concentration, enzyme concentration, product reactions, cofactors, and pH, among other factors, have profound effects on enzyme activity. In addition, sources (bacterial versus fungal) and activity of enzymes differ markedly. Tricarico et al. (2007) examined the effects of an *Aspergillus oryzae* extract containing -amylase activity (950 dextrinizing units (DU)/kg of DM) on performance and carcass characteristics of finishing beef cattle fed roughage source (alfalfa hay vs. cottonseed hulls). In steers fed cottonseed hulls, supplemental -amylase increased ADG through d 28 and 112 as a consequence of increasing DMI and efficiency of gain during the initial 28-d period. In agreement, Tricarico et al. (2007) reported that in crossbred heifers the alpha-amylase supplementation increased DMI and ADG during the initial 28 d.

Instead of feed additives, feeding strategies such as the supplementation of ruminant animal diets with fat, oils, and whole seeds has also been proposed to increase the feed efficiency (Hess et al., 2008). Because of its caloric density, the primary function of fat in diets consumed by ruminants is to provide energy (NRC, 2001). Another reasons for supplementing ruminant diets with fat has been a decrease in DMI without decreasing ADG, dust reduction, fines elimination, and pelleting aiding (Byers and Schelling, 1988). Although tallow is a supplemental fat source commonly fed to finishing cattle, the use of other supplemental fat sources, such as whole oilseeds, in finishing cattle diets has been explored. Thus, Montgomery et al. (2005) reported that growth performance of finishing cattle fed supplemental fat, such as tallow or as dried full-fat corn germ was similar. In addition, LaBrune et al. (2002) reported that growth performance of steers fed finishing diets containing supplemental fat, as tallow or ground linseed, was not different. However, contrary to expectation, Maddock et al. (2004) fed whole or processed (rolled or ground) linseed, included at 8% of diet DM, reported significant increases in gain and gain efficiency and no differences in DM intake, when compared with a corn-based control diet. However, actually, oil/fat oilseeds prices are also high, and their levels of inclusion (mainly oils) in ruminant diets use needs to be restricted because they are likely to cause digestive problems, interfere with ruminal fermentation, influence post-ruminal digestibility, cause difficulties in pelleting process of concentrate, or reduce the DMI due to palatability problems.

2.5.3. Increasing intensive beef production efficiency by using alternative feed ingredients

Depending on the cost of more traditional feedstuffs, alternative feeds (e.g. by-products) often provide an opportunity to reduce the cost of total diet while maintaining or improving animal performance. In fact, the use of industrial by-products in the ruminant diets has been a common

practice for decades. The unavailability of forage in Catalonia gives even more importance to industrial by-products as sources of nutrients for ruminants. By-products may be defined as left over feedstuffs from other agricultural industries or non-agricultural industries. By-products are often low-priced feeds, which could replace grain or more expensive protein supplements in the feeding of growing bulls. Barley fibre, barley protein, and wet distillers soluble are most commonly used, and have been extensively studied during the last decades. For example, in the North America there are studies with distillers grains derived from wheat (Fisher et al. 1999; Mustafa et al., 2000), corn (Larson et al., 1993; Lodge et al., 1997) used in the ruminants diets. However, while these by-products can contribute to reducing costs of feeding, attention must be given to the amount that can be reasonably included in the ration (Lodge et al., 1997), as well as, to the nutrient content of each by-product, its composition variation from load to load (Guiroy et al., 2000), its availability (by geographical region and principal producing countries), its transportation costs relative to product value, and its rapid spoilage, and lack of preservation facilities.

In recent years, as a result of growing interest in alternate sources of energy, including biofuels, crude glycerin has appeared as a potential by-product used in ruminants diets. The European Commission (EC) is using both legislation and formal directives to promote biofuel production with the objective to incorporate a minimum of 10% biofuel by 2020 in total transport fuel use. The major feedstock for EU biodiesel production is rapeseed oil, while bioethanol is generally produced using a combination of sugar beets and wheat. The National Biodiesel Board has projected an annual production of biodiesel in 2007 of 1,703 million litres, a sharp increase from less than 379 million litres prior to 2005 (National Biodiesel Board, 2008). As biodiesel production increases, so does production of the primary by-product, crude glycerin. In general, production of 100 kg of biodiesel yields approximately 10 kg of crude glycerin (Dasari et al., 2005), which is impure and of low economic value. The impurities include methanol, soaps, and a variety of elements such as calcium, magnesium, phosphorous, or sulphur (Thompson and He, 2006). It has been reported that glycerin contains from 65% to 85% of the glycerol (González-Pajuelo et al., 2005). The wide range of the purity values can be attributed to different crude glycerin purification methods used by the biodiesel producers, and the different feedstock used in biodiesel production. Because there is a glut of this impure glycerol, there have been many investigations into alternative uses for it. Combustion, composting, animal feeding (recognized as emulsifying and stabilizing agent, thickness and gelling agent, Council Directive 70/524/EC), thermo-chemical conversions and biological conversion methods for glycerol usage and disposal have all been proposed. For example, Johnson and Taconi (2007) reported that combustion of crude glycerol is a method that has been used for disposal. However, this method is not economical for large producers of biodiesel. It has also been suggested that glycerol can be composted (Brown, 2007) or used to increase the biogas production with anaerobic digesters (Holm-Nielsen et al., 2008). DeFrain et al. (2004) attempted to feed biodiesel-derived glycerol to dairy cows in order to prevent ketosis, but found that it was not useful. Lammers et al. (2008) studied supplementing the diet of growing pigs

with crude glycerol. This study found that the metabolizable to digestible energy ratio of glycerol is similar to corn or soybean oil when fed to pigs. Therefore, the study concludes that crude glycerin can be used as an excellent source of energy for growing pigs, but also needs to be used with caution because little is known about what the impacts of impurities of the glycerol can have on animal health. Cerrate et al. (2006) reported some success when feeding glycerol to broiler chickens. Birds fed 2.5 % of 5% glycerin diets had higher breast yield than the control group, but the authors mentioned that caution should be exercised with the glycerin use because there is still concern about methanol impurities within the glycerol. Hence, intuitively, the use of crude glycerin from the biodiesel production process, as an energetic ingredient in growing bulls, might be a good strategy to reduce the feeding cost and increase the intensive beef production efficiency.

3. SUMMARY

In recent years, efforts to improve the efficiency of intensive beef production in Spain have been further investigated. One way in which efficiency of intensive beef production might be improved is by enhancing the end carcass and meat quality. Studying the effect of different pre-slaughter factors (related to animal, farm, transportation, and animal handling at the slaughterhouse) could allow the proposal of pre-slaughter management, and technical decisions to improve carcass and meat quality (mainly decreasing the incidence of high ultimate pH), and could increase the efficiency of intensive production systems. Also castration, as a management practice, could improve the end carcass and meat quality, mainly improving the grade of backfat, and meat tenderness and intramuscular fat content. However, the practices of castration illustrate the delicate balance between arguments about animal welfare and some management practices in the farm. The supplementation of seeds rich in omega-3 fatty acids in the diet of ruminant to obtain a functional food, could achieve the differentiation of the meat in the food marked, and create fidelity towards beef meat. However, it is important to consider linseed prices and its availability, as well as the certification and legislation in order to implement this strategy successfully. And finally another way to increase the beef production efficiency could take into account the reduction in the cost of beef production during the finishing phase through feeding animals with crude glycerin, as a energy ingredient, although this strategy is highly dependent on biodiesel production, alternative uses of glycerin, as well as the on cereal prices.

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CHAPTER II: OBJECTIVES

CHAPTER II:

OBJECTIVES

The main objective of the present thesis was to study feeding and management strategies to increase intensive Holstein beef production efficiency. The specific strategies were:

1. To evaluate the effects of different pre-slaughter factors on the incidence of high ultimate meat pH and extreme carcass bruises, in order to suggest any proposal pertaining to management, technical or economical decisions, which could lead to improvements on carcass and meat quality, and to increase the beef production output.

2. To improve the carcass backfat classification, and meat quality characteristics, during the finishing phase, by castrating Holstein bulls, without affecting the performance, in order to increase the beef production output.

3. To achieve the differentiation of final quality of meat in the food market, by supplementation of specific feed ingredients in the ruminant diet, while maintaining or improving animal performance, rumen fermentation, in order to increase the beef production output.

4. To reduce the cost of diet by feeding animals an industrial by-product, while maintaining or improving animal performance, rumen fermentation, metabolism, and carcass and meat quality, in order to reduce the beef production input.

To achieve these specific strategies, four studies were conducted:

Study 1: Association between animal, transportation, slaughterhouse practices, and beef extreme carcass bruises and ultimate meat pH.

Study 2: *Burdizzo* pre-pubertal castration effects on performance, behaviour, carcass characteristics and meat quality of young Holstein bulls fed high-concentrate diets.

Study 3: Increasing the amount of omega-3 fatty acid of meat from intensively fed young Holstein bulls through nutrition.

Study 4: Effects of glycerin supplementation on performance and meat quality in young

Holstein bulls fed high-concentrate diets.

CHAPTER III: ASSOCIATION BETWEEN ANIMAL, TRANSPORTATION, SLAUGHTERHOUSE PRACTICES AND BEEF EXTREME CARCASS BRUISES AND ULTIMATE MEAT pH

CHAPTER III:

ABSTRACT

The objective of this study was to evaluate the influence of factors related to animal, farm, transportation, and animal handling at the slaughterhouse, as well as their interactions, on beef ultimate meat and extreme carcass bruises. A total of 5,494 cattle (343 ± 45 d of age) from 181 different Catalonia farms were surveyed during 3 seasons (spring, summer, and winter), and a total of 25 pre-slaughter variables were recorded. Ultimate meat pH was measured at the M. *longissimus* at 24h *post-mortem* and extreme carcass bruises were registered at 24h *post-mortem.* After a variable selection procedure, mixed-effects logistic regression model was conducted with fixed and random variables to identify the main factors, and their interactions, affecting ultimate meat pH and extreme carcass bruises. Average incidences of ultimate meat pH greater than 5.8 and 6.0 were 13.89% and 4.02%, respectively, and the incidence of extreme carcass bruises was 2.43%. Although several factors significantly affected ultimate meat pH and carcass bruises incidence, the variability explained by the models (4.1% and 13% respectively) was too low to allow the proposal of management, technical, and economic decisions to improve meat quality in the Spanish market.

Keywords: Beef, Meat pH, Extreme Carcass Bruises, and Pre-slaughter Management

CHAPTER III:

1. INTRODUCTION

Beef with ultimate meat pH above 6.0 represents a meat quality problem, and is undesirable for human consumption (Corstiaensen et al., 1981; Viljoen et al., 2002; Wulf et al., 2002; Pipek et al., 2003). Furthermore, beef meat with high ultimate pH causes important industry economic losses. For example, the Spanish meat industry penalizes carcass price with discounts between 30 and 60% when ultimate meat pH is greater than 5.8. The main problems of ultimate meat pH above 6.0 are a dark red colour (Bartos et al., 1993; Kreikemeier et al., 1998; Mounier et al., 2006), increased tenderness variation (Silva et al., 1999), increased water holding capacity (Apple et al., 2005; Zhang et al., 2005), poor palatability (Viljoen et al., 2002; Wulf et al., 2002), and growth of microorganisms to unacceptable levels with development of off-odours, and often slime formation (Gardner et al., 2001). After exsanguination, as soon as blood flow ceases, muscle cells are subjected to hypoxia. With the development of hypoxic conditions, the first modification is very likely a rapid decline of the pH as a result of lactic acid produced from anaerobic glycolysis. About 45 μmol of glycogen is needed to lower the pH of 1 kg of muscle from 7.2 to 5.5 (Immonen et al., 2000). Presumably, *post-mortem* glycolysis and muscle pH decline is stopped, under normal carcass chilling conditions, when muscle pH declines to approximately 5.45, and this low pH inhibits the activity of glycolytic enzymes, or when muscle glycogen concentrations are depleted. Glycogen concentration shows an inherently variable nature dependent on breed (Gardner et al., 2003), gender (Shackelford et al., 1994; Hoffman et al., 1998), nutritional status of animal and feed intake (Schaefer et al., 1997; Geay et al., 2001; Gardner and Thompson, 2003; Bee et al., 2006), live weight (Smith and Dobson, 1990), temperament (Gardner et al., 2003; King et al., 2006), electrical stimulation, the type of muscle, and fibres, and physical exhaustion and physiological pre-slaughter stress (Nockels et al., 1996; Immonen and Puolanne, 2000). Various pre-slaughter stress factors have been reported as responsible for glycogen depletion: time and handling during transportation from farm to slaughterhouse (Schaefer et al., 1997; Hoffman et al., 1998; Arthington et al., 2003; Honkavaara et al., 2003), waiting time at slaughterhouse (Warriss, 2003), climatic factors (Kreikemeier et al., 1998; Silva et al., 1999), social disruption (Whythes et al., 1979; Apple et al., 1995; Hambrecht et al., 2005), and the novelty of pre-slaughter environment (Hambrecht et al., 2005; Mounier et al., 2006). Although, these cited studies have reported the effect of different factors on glycogen concentration and beef ultimate meat pH, there is a lack of information concerning the effect of the interaction between these factors and ultimate meat pH, as well as the impact of each factor on the proportion of ultimate meat pH variability.

Carcass extreme bruises, described as a huge tissue injury with rupture of the vascular supply and accumulation of blood and serum, represents also an important economic loss for Spanish meat industry. Producers are paid at lower rate as a consequence of downgraded carcasses, the reduction of carcass weight, and the trimming labour. In general, extreme carcass bruises might occur on the farm, during the transport, and at the slaughterhouse. Factors which might cause bruising include: the physical environment (Grandin, 1993), the social environment (Tarrant et al., 1988), animal temperament and the amount of handling the animals have experienced in the past (Vowles, 1977), and the behaviour of the handlers (Mounier et al., 2006). Very little is known of the extend of extreme carcass bruises in commercially slaughtered beef, and even less about the interaction between pre-slaughter factors related to animal, farm, transportation, and animal handling at the slaughterhouse, and beef extreme carcass bruises.

Therefore, the objective of this study was to evaluate the influence of some factors related to animal, farm, transportation, and animal handling and behaviour at the slaughterhouse, as well as their interactions, on beef ultimate meat pH and extreme carcass bruises.

2. MATERIALS AND METHODS

2.1.Data collection

A total of 25 variables related to animal, farm, transportation, and animal handling at the slaughterhouse were recorded during 3 seasons (summer, spring, and winter) of year 2005 (Table 1, 2 and 3). The location and number of animals from 181 representative Catalonia farms were recorded. The truck identification, the number of animals loaded in the truck, the stocking density in the truck compartments, whether unacquainted animals were mixed (different pen and/or origin) or whether unacquainted animals were mixed with different gender, the departure time from the farm, the arriving time at slaughterhouse, and the transportation distance from farm to slaughterhouse, were recorded. Average daily temperature of the transportation day was obtained from the Barcelona Weather Records (Servei Meteorològic de Catalunya, Spain).

All cattle were slaughtered in the main commercial slaughterhouse of Barcelona. The plant operated from 0600 to 1400h on Monday, from 0400 to 1200h on Tuesday, Wednesday, and Thursday, and from 0.00 to 8.00h on Friday. Upon arrival to the slaughterhouse, animals were moved through a corridor, and distributed to different pens (from 1 to 82) according to gender, farm origin, finishing group size, and age (greater or lower than 365 d of age). Cattle were slaughtered after stunning by a captive bolt, suspended by a hind leg, and exsanguinated. The arrival time at slaughterhouse, the waiting time at slaughterhouse, the number of animals per pen, the stocking density (animal per m^2), the number of animals slaughtered daily, and the daily ratio between males and females slaughtered, were recorded.

Table 1. Means standard deviation (SD), standard error (SEM), maximum and minimum values for the continuous independent variables used in the analysis

Item	Total observations	Mean	SD	SEM	Minimum	Maximum
Animal						
Age (days)	3316	343.8	44.84	0.77	208.0	703.0
Farm						
Number animals per farm	4190	249	174.4	2.69	30	700
Transport						
Number of animals in the truck	1571	31	9.8	0.25	10	52
Number of animals in a compartment of the truck	1427	9.8	2.53	0.07	2.0	18.0
Stocking density (animal per m^2)	1346	0.8	0.23	0.001	0.2	1.8
Distance (Km)	4913	134.9	50.87	0.72	40.0	550.0
Duration (h)	2425	3.7	1.98	0.04	1.2	15.7
Slaughter day temperature (ºC)						
Maximum	5494	20.1	8.66	0.11	6.9	35.4
Minimum	5494	11.5	6.67	0.09	2.8	24.6
Slaughterhouse						
Animal per pen	5418	10.3	3.37	0.05	1.0	25.0
Stocking density (animal per m^2)	5381	0.31	0.098	0.001	0.02	0.78
Waiting time (h)	5456	12.3	6.06	0.08	0.9	37.2
Number of slaughtered animals per day	5394	377	188.5	2.57	0	724
Ratio male/female	5284	2.5	3.55	0.05	0.0	42.5
Carcass characteristics						
Ultimate pH of M. *longissimus*	5494	5.67	0.16	0.002	5.48	6.98
Hot carcass weight (kg)	5480	248	37.51	0.51	116	466

2.2. Measurements and Sample Collection

From 182 different pens, animal behaviour was registered on different waiting times at slaughterhouse. Whereas animal social behaviour (sexual interactions) was scored using a 3 continuous behaviour sampling of 15 min each one, the general activities of animals were scored using 9-scan sampling of 10s at 5 min interval (Mounier et al., 2005). Sexual interactions included attempted mounts (head on the back of another animal), and completed mounts. The general activity of animals were resting, standing, drinking, and ruminating. For each pen, the proportion of animals in the same activity was calculated.

Animal gender, breed, age at slaughter, hot carcass weight, backfat and conformation classification (EU classification system into 1.2.3.4.5 and into (S) EUROP categories by EU Regulation nº 1183/2006, respectively), were also recorded. The conformation class designated by the letter “E” (Excellent) describes carcasses with all profiles convex to super-convex, and with exceptional muscle development, whereas the conformation classified as “U” (Very good) describes carcasses with profiles on the whole convex, and with very good muscle development. The carcasses classified as “R” (Good) present profiles on the whole straight, and good muscle development. Carcasses classified as “O” (Fair) present profiles straight to concave, and with

average muscle development, whilst carcasses classified as “P” (Poor) present all profiles concave to very concave with poor muscle development. In addition, the degree of backfat describes the amount of fat on the outside of the carcass and in the thoracic cavity. While the class of backfat that classifies as 1 (Low) describes none to low fat cover, the class of backfat classified as 5 (Very High) describes an entire carcass covered with fat and with heavy fat deposits in the thoracic cavity.

Table 2. Frequencies for the categorical independent variables used in the analysis related with season, animal, transport and slaughterhouse

	Total observation	n[1]	Frequency (%)
Season	5494		
Spring		1964	35.75
Summer		1630	29.67
Winter		1900	34.58
Animal			
Gender	5480		
Male		3402	62.08
Female		2078	37.92
Breed type	4198		
Asturiana		23	0.55
Belgium		2	0.05
Charolais		3	0.07
Fleckvieh		665	15.78
Holstein		2179	51.72
Limousine		21	0.50
Mixed		1103	26.54
Montbelier		16	0.38
Parda Alpina		186	4.41
Breed group	5494		
Holstein		2179	39.66
Crossbreed		3315	60.34
Transport			
More than one origin in the truck	4778		
No		2610	54.61
Yes		2168	45.39
More than one gender in the truck	4778		
No		1266	26.29
Yes		3512	73.71
Arrival time, h	5443		
0700-1800		2040	37.48
1900-0600		3403	62.52

[1]n= number of animals corresponding to the criterion

Although, 12 different breed types of animals were registered, over 51% of the animals were Holstein. Therefore, data corresponding to breed were classified into two groups: Holstein and crossbreeds. The same investigator collected all data during all the observation dates.

Ultimate meat pH was measured in the M. *longissimus* (LM) of 5,494 carcasses between 14th and 15th rib, on the left side at a depth of 4 cm, with a portable pHmeter (CRISON pH25, CRISON Instruments SA, Spain) equipped with a xerolyt electrode. The pHmeter was calibrated

with pH 4 and pH 7 standard solutions (CRISON Instruments SA, Spain) at a temperature 2ºC every 50 measurements. Carcasses with ultimate pH lower than 5.8 were classified into normal quality, and carcasses with ultimate pH values greater than 5.8 were classified as a devaluated meat quality, in accordance to Spanish meat industries criteria, and to a recent study (Viljoen et al., 2002). Average incidence of extreme bruises was recorded from 3,864 carcasses at 24 h *post-mortem*. A bruise severity score of extreme implicates a loss of edible part.

Animals were managed following the principles and specific guidelines of the IRTA Animal Care Committee.

Table 3. Frequencies for the categorical independent variables used in the analysis related with carcass characteristics

	Total observation	N[1]	Frequency (%)
Carcass characteristics			
Bruises (%)	3864	94	2.43
pH of LM	5494		
pH < 5.8		4731	86.11
pH ≥5.8		763	13.89
pH < 6		5273	95.98
pH ≥ 6		221	4.02
Conformation	5478		
E		13	0.23
U		371	6.77
R		2415	44.09
O		2582	47.14
P		97	1.77
Carcass backfat	5477		
1		38	0.70
2		1165	21.27
3		4274	78.03

[1]n=number of animals corresponding to the criterion

2.3. Statistical analyses

The first step for meat ultimate pH statistical analyses was the selection of variables to be included in the model. A Pearson correlation analysis was performed to identify pairs of continuous variables that contained essentially the same information, avoiding multicollinearity in the model. When pairs of correlated ($R^2 > 0.10$) variables were found, only one was selected for inclusion in the model based on biological relevance. A Chi-square-test was conducted to test the effects of categorical variables on meat ultimate pH. Finally, for the ultimate meat pH data, the selected variables were season (spring, summer, or winter), gender, mixing unacquainted animals from different origins in the truck, mixing more than one gender in the truck, transport distance (km), arrival hour at the slaughterhouse (h), stocking density at slaughterhouse (animal per m^2), waiting time at the slaughterhouse (h), backfat (from 1 to 5) and conformation carcass classification (SEUROP). In order to facilitate the interpretations, continuous variables were categorized into four discrete classes according to their quartile distribution. The plot of standardised residuals versus

predicted values suggested that residuals were not normally distributed at all levels of the predictors, and at all combinations of predictors in the model. In an attempt to attain a normal distribution, the natural log of meat ultimate pH and meat ultimate $pH^{½}$ were assessed. Because the residuals continued clearly not normally distributed, a mixed-effects logistic regression analysis was considered. Thus, the meat ultimate pH was analyzed as a binomial response variable with values of 0 (pH < 5.8) and 1 (pH ≥ 5.8). The odds ratio (OR) were calculated, and the no statistically significant variables and interactions between main factors were removed from the model. The final statistical model included gender, waiting time at the slaughterhouse, backfat carcass classification, the interaction between gender and stocking density at slaughterhouse, and the interaction between gender and backfat carcass classification, as fixed effects, and truck, as random effect. For extreme carcass bruises incidence, the mixed-effect logistic regression model included season (spring, summer or winter), gender, transport distance (km), stocking density in the truck (animal per m^2), mixing unacquainted animals from different origins in the truck, waiting time at the slaughterhouse (h), stocking density at slaughterhouse (animal per m^2), and hot carcass weight (kg), as fixed effects, and truck, as random effect. To analyze behaviour data, pen was considered the experimental unit. The sexual behaviour data were analyzed using a Poisson regression. The model included the gender, the waiting time, and stocking density at the slaughterhouse, and their interactions, as fixed effects, and the pen as random effect. The number of animals in each pen was included as a weight variable. The Incidence Relative Ratio (IRR) was calculated. The general activities of animals were averaged for each pen, transformed into natural log, to achieve a normal distribution, an analyzed using mixed-effects ANOVA with the same model as sexual behaviour data.

3. RESULTS AND DISCUSSION

The incidence of meat ultimate pH lower and greater than 5.8 associated with each categorical variable studied are presented in Table 4 and 5, and the logit of the outcome of logistic regression model are presented in Table 8. In addition, the average incidence of extreme carcass bruises associated with each categorical variable studied is presented in Table 6 and 7, and the logit of the outcome of logistic regression model are presented in Table 9.

The incidence of ultimate meat pH was greater than 5.8 and 6.0 for 13.89% and 4.02% of cattle, respectively (Table 3). In contrast to our results, Kreikemeier and Unruh (1993) after analyzing 8,000 heifers carcasses at a commercial slaughterhouse in USA, reported that incidence of ultimate meat pH greater than 5.8 was only 1.7%, and in France, Mounier et al. (2006) reported that ultimate meat pH greater than 6.0 was 2.79%. These differences among studies could be attributed to the different fattening beef production systems. Thus, whereas in France late-maturing breeds are raised to slaughter weights between 500 and 600 kg feeding silage supplemented with concentrates, in Spain, early-maturing breeds are fed with concentrates and straw (5% to 10% of

the total diet) *ad libitum* from 12 to 14 weeks of age to slaughter weights of 460-500 kg. For example, in our study, animals were slaughtered at the age of 343 ± 45 d, with an average hot carcass weight of 248 ± 37.5 kg (Table 1). Moreover, the 62.1% of cattle slaughtered were males, and nearly 50% of them were Holstein (Table 2). Furthermore, average incidence of extreme carcass bruises was 2.43%. Similarly, McKenna et al. (2002) in the National Beef Quality Audit-2000 reported an incidence of extreme bruises from 2.6 to 6.5%.

Results from logistic regression model indicated that ultimate meat pH only tended to be affected by gender (P = 0.08), being the incidence of ultimate meat pH ≥ 5.8 greater in males than in females (OR= 2.15), in agreement with Hoffman et al. (1998), and Shackelford et al. (1994). Furthermore, it is important to consider that gender was correlated with breed type (R^2= 0.12; P < 0.001), and also with farm capacity (R^2= 0.18; P < 0.001). In that sense, nearly 50% of males surveyed in the present study were Holstein, and 50% of them were housed in farms holding about 140 ± 3.2 animals. In consequence, gender, breed type, and farm capacity effects were confounded in the current study, difficulting to support the results observed by King et al. (2006), and Önenç (2004), concluding that breed type could affect temperament, stress responsiveness, and in consequence, beef carcass quality. In addition, the average incidence of carcass bruises was 45% lower (P < 0.001) in females than in males (OR = 0.44).

Cattle were moved from farms to slaughterhouse with 34 different trucks, with 3 of them transporting around 57% of the total animal involved in the survey. Transportations were mainly (62%) conducted between 1900 and 0600 h (Table 2), and the temperature data during transportation ranged from 3ºC in winter to 35ºC in summer (Table 1). The average number of animals per truck was 31.15 ± 9.88, and the average of stocking density during transport was 0.82 ± 0.23 animals per m^2 (Table 1). This stocking density is in accordance with the density recommended by European Community directives (64/432/EC and 93/119/EC) for 500 kg cattle. The stocking density during the transport affected (P < 0.01) the extreme carcass bruises, increasing the incidence above 45% (OR= 1.45), when stocking density at the truck was up to 1.30 animal per m^2. In agreement, Eldridge and Winfield (1988) indicated that carcass bruises increased as stocking density in the truck increased above 0.72 animals per m^2.

Table 4. Incidence of ultimate meat pH related with season, animal, transport and slaughterhouse

		pH < 5.8		pH ≥ 5.8	
Item	Total observation	n[1]	Frequency (%)	n[1]	Frequency (%)
Season	5494				
Spring		1697	86.68	261	13.32
Summer		1374	84.29	259	15.71
Winter		1660	87.37	243	12.63
Animal					
Gender	5480				
Male		2821	82.92	581	17.08
Female		1896	91.24	182	8.76
Breed group	5494				
Holstein		1809	83.02	370	16.98
Other breeds		2922	88.14	393	11.86
Transport	4778				
More than one origin in the truck					
No		1845	85.10	323	14.90
Yes		2278	87.76	332	12.24
More than one gender in the truck	4778				
No		2978	84.81	532	15.19
Yes		1133	89.49	135	10.51
Distance (km)	4913				
≤100		1025	87.46	147	12.54
101-135		1007	85.41	175	14.59
136-150		1072	84.88	191	15.12
≥151		1115	86.10	180	13.90
Stocking density (animal per m^2)	1346				
<1.29		553	87.36	80	12.64
≥1.30		641	89.90	72	10.10
Duration (h)	2425				
≤ 2.10		452	86.59	71	13.41
2.11-2-75		401	86.24	64	13.76
2.76-3.45		676	85.25	118	14.75
≥3.46		552	85.85	91	14.15
Slaughterhouse					
Arrival time	5443				
0700-1800		1702	83.43	338	16.57
1900-0600		2983	87.66	420	12.34
Stocking density (animal per m^2)	5381				
≤0.26		1203	84.24	225	15.76
0.27-0.30		877	85.23	152	14.77
0.31-0.37		1267	84.81	227	15.19
≥0.38		1290	90.21	140	9.79
Waiting time (h)	5456				
≤8.16		1224	89.15	149	10.85
8.17-11.86		1198	88.02	163	11.98
11.87-15.80		1201	87.92	165	12.08
≥15.81		1070	78.91	286	21.09

[1]n=number of animals corresponding to the criterion

Table 5. Incidence of ultimate meat pH related with carcass characteristics

		pH < 5.8		pH ≥ 5.8	
Item	Total observation	n[1]	Frequency (%)	n[1]	Frequency (%)
Carcass characteristics					
Extreme carcass bruises (%)	3864	77	2.29	17	3.35
Conformation	5478				
E		10	83.33	3	16.67
U		315	84.91	56	15.09
R		2153	89.15	262	10.85
O		2181	84.47	401	15.53
P		66	68.04	31	31.96
Carcass backfat	5477				
1		22	57.89	16	42.11
2		917	78.71	248	21.29
3		3786	88.58	488	11.42
Hot carcass weight (kg)	5480				
≤222.5		1165	86.49	182	13.51
223-245		1166	87.21	173	12.79
245.5-269.5		1183	84.56	219	15.44
≥270		1200	86.21	192	13.79

[1]n=number of animals corresponding to the criterion

In Catalonia, loading animals from different origins in the same truck is a common practice. In the present study, 54.6% of trucks mixed animals from different farms, and 26.3% of trucks mixed animals with different gender, during transportation (Table 2). However, mixing unacquainted animals from more than one origin in the same truck or from different genders did not affect ultimate meat pH, and in contrast to expected, mixing unacquainted animals from more than one origin or pen in the same truck decrease carcass bruises ($P < 0.01$; OR= 1.54). This could be due in part, to the fact that mixing unacquainted animals in the same compartment during transport was avoided in 80% of cases, limiting the interactions (Mounier et al., 2006). In addition, since the transported animals probably were not be able to interact (fight or mount) in the truck, mixing unacquainted animals in the transport seems not to be a primary cause of high incidence of ultimate meat pH. Also, the transport distance, which was correlated with transportation hours (R^2= 0.27; $P < 0.001$), did not affect ultimate meat pH and extreme carcass bruises, suggesting that physical activity and psychological stress associated with transportation lower than 151 km or 3.46 h (Table 1) were probably too low to exert negative effects on ultimate meat pH. To reinforce this hypothesis, the European Union Scientific Committee on Animal Health and Animal Welfare (2001) reported that transport causes loss of weight, degradation of meat quality, and alterations in some physiological stress parameters mainly over 8 h. Other authors (Bartos et al., 1993; Fernandez et al., 1996; María et al., 2003) reported no significant relationship between the incidence of high ultimate meat pH and transport duration in animals transported less than 6 h. Additionally, Honkavaara et al. (2003) and Whithes et al. (1979) also did not report significant relationship between the extreme carcass bruises and transport duration.

At the slaughterhouse, animals were housed in different pens, in groups of 10.3 ± 3.36 animals with 0.31 ± 0.09 animals per m^2, during an average waiting time of 12.3 ± 6.06 h with a maximum of 37.2 h (Table 1). The waiting time at slaughterhouse affected ($P < 0.001$) ultimate meat pH. Our results indicated that while the odds ratio of meat ultimate pH slightly increased with waiting time at slaughterhouse (OR= 1.11 and 1.12, for 8.17 to 11.86 and 11.87 to 15.80 h, respectively), after 15.80 h of waiting time at slaughterhouse, the odds ratio of meat ultimate pH doubled (OR= 2.19, Table 8). These results are in disagreement to those reported by Mounier et al. (2006), who concluded that in bulls, ultimate meat pH decreased accordingly when waiting time at slaughterhouse was 1, 17 or 40 h, and suggested that bulls should stay at slaughterhouse more than 17 h to avoid high ultimate meat pH. Furthermore, waiting time at slaughterhouse affected ($P < 0.05$; Table 6 and 9) the incidence extreme carcass bruises. Present results indicated that whilst the incidence of extreme carcass bruises increased until 11 h of waiting time at slaughterhouse, after 11.8 h of waiting time at slaughterhouse, the incidence of extreme carcass bruises was reduced (Table 6), in contrast to that reported by McNally and Warris (1996). A statistically significant interaction ($P < 0.01$) between stocking density at slaughterhouse and gender was found (Table 8), indicating that while ultimate meat pH ≥ 5.8 of males increased as stocking density increased, in females remained constant. Thus, increasing stocking density at slaughterhouse could have a greater impact on the incidence of meat with meat ultimate pH≥ 5.8 in males than in females, in accordance to Fisher et al. (1997), probably because males have greater physical activity and physiological stress than females (Kreikemeier et al. 1998). In contrast, the stocking density at slaughterhouse did not affect the incidence of extreme carcass bruises.

Backfat carcass classification affected ($P < 0.001$) ultimate meat pH. The OR of ultimate meat pH ≥ 5.8 decreased from 0.37 to 0.18 (Table 8) as carcass backfat classification increased from 1 to 2, and from 1 to 3, respectively, in agreement with Kreikemeier et al. (1998). In fact, the animals corresponding to “1” backfat classification presented and incidence of ultimate meat pH ≥ 5.8 up to 42%. Furthermore, an interaction between gender and backfat carcass classification tended to affect ($P = 0.08$) ultimate meat pH ≥ 5.8, indicating that females with lean carcasses were more susceptible to have a ultimate meat pH above 5.8 than males (Table 8). However, the ultimate meat pH ≥ 5.8 in carcasses with backfat classified as “2” and “3” was 5.88 and 2.08 times greater in males than in females, respectively (Table 8). It is important to take into account that carcass backfat is also an important determinant of glycolytic rate, and ultimate meat pH, largely due to the effect of temperature on glycolysis. Subcutaneous fat cover is thought to act as an insulator, retarding the rapid rate of temperature decline and consequently preventing the high ultimate meat pH as a result of enzyme inactivation. In fact, Koohmaraie et al. (1988) reported that the effect of temperature on pH is even more eident at 0ºC. At 0ºC, in the case of defatted carcasses, reduction in pH is not completed even after 24h *post-mortem*, while in the case of control carcasses; the ultimate meat pH was attained after 9 h *post-mortem*.

Similarly, animals with backfat classification as “1” presented ($P < 0.001$) an incidence of extreme carcass bruises up to 11.11%. These results suggested that small carcasses with poor muscle development and none to low backfat are probably related to physical exhaustation or health problems. The other variables studied, and the interactions between them, did not affect the ultimate meat pH, and the extreme carcass bruises.

Despite the range of data considered in the present study, and the statistical significance of some factors included in the logistic regression model, ultimate meat pH variability explained by the model was extremely low (R^2 = 4.9%), and carcass bruises incidence variability explained by the model was 13%. This makes not feasible any proposal pertaining to management, technical or economical decisions, such as optimizing stocking densities, and waiting time at the slaughterhouse, and applying different handling practices between males and females in order to reduce carcass and meat quality problems. In agreement with the present study, Mounier et al. (2006) explained a low proportion of ultimate meat pH variability (R^2 between 1.31% and 7.99%) when studying the impact of the conditions from farm to slaughter. Moreover, the measurement of meat ultimate pH could be an insensitive measure of physical exhaustion and physiological pre-slaughter stress. Gardner et al. (2001) suggested that muscle glycogen in the live animal must be depleted to levels below about 45 μmol/kg before carcass meat ultimate pH decreases, being a possible explanation for the low R^2 observed in the present study.

However, the study of sexual interactions, as well as resting and ruminating general activities, might explain the relationship between some slaughterhouse variables and the incidence of high ultimate meat pH and extreme carcass bruises. Intuitively, it was though that immediately after arriving at slaughterhouse, the animals should exchange more agonistic behaviour. It is registered that animals from the same one-farm origin pens are transported and slaughtered at different days as a result of different final body weights, in groups of 8-9 animals. Therefore, it is probably that those animals establish a new dominance hierarchy once at the slaughterhouse. In fact, Mounier et al. (2006) stated that bulls are more stressed during slaughter when they have been separated from their usual social partners at the slaughterhouse. In the present study, average incidence of animal mountings per pen at the slaughterhouse was 0.18 ± 0.02-times/5 min, being the incidence rate ratio (IRR) of mountings 4.54 times greater ($P < 0.001$) in males than in females. In contrast to expected, after 15.80 hours of waiting time, the incidence of mountings in males was 3-times greater ($P < 0.001$; IRR= 3.32) than during the first hours after arriving at slaughterhouse; whilst in females the incidence of mountings remained constant throughout waiting time at the slaughterhouse (Table 10). These results probably might explain the increase incidence of high ultimate meat pH over 21% after 15.81 h of waiting time at the slaughterhouse. Instinctively, animals that display a greater incidence of mounting behaviour are more likely to incur bruising during pre-slaughter handling, and to present high ultimate meat pH. In fact, Voisinet et al. (1997) reported that temperament was significantly correlated with the incidence of high ultimate pH as determined by subjective colour assessment in a study involving 306 cattle.

Table 6. Incidence of extreme carcass bruises related to animal, transport and slaughterhouse

Item	Total observation	N[1]	Frequency (%)
Animal			
Gender class	3850		
Male		75	3.04
Female		19	1.37
Breed group	3864		
Holstein		57	3.75
Others		37	1.58
Transport			
More than one origin in the same truck	3423		
No		53	2.97
Yes		27	1.65
More than one gender in the same truck	3385		
No		62	2.40
Yes		17	2.12
Distance (km)	3369		
<100		16	1.81
101-135		26	3.17
136-150		25	3.17
≥151		24	2.73
Stocking density (animal per m^2)	1346		
<1.29		59	1.58
≥1.30		25	3.51
Duration (h)	1566		
< 2.1		7	1.96
2.1-2-75		1	0.30
2.76-3.45		20	3.96
≥3.46		8	2.13
Slaughterhouse			
Arrival hour	3849		
0700-1800		32	2.01
1900-0600		62	2.74
Stocking density (animal per m^2)	3791		
< 0.26		20	2.15
0.27-0.30		31	3.04
0.31-0.37		22	2.31
≥0.38		19	2.14
Waiting time (h)	3864		
<8.16		26	2.59
8.17-11.86		34	3.61
11.87-15.80		17	1.77
≥15.81		17	1.78
Number of animal slaughtered	3738		
Monday		17	2.11
Tuesday		15	2.25
Wednesday		10	2.10
Thursday		16	2.25
Friday		36	3.00

[1]n= number of animals corresponding to the criterion

Table 7. Incidence of extreme carcass bruises related with carcass characteristics

Item	Total observation	n[1]	Frequency (%)
Carcass characteristics			
Carcass pH	3864	77	2.29
pH < 5.8		17	3.35
pH ≥ 5.8			
pH < 6.0		86	2.32
pH ≥ 6.0		8	4.85
Conformation	3847		
U		2	0.81
R		26	1.49
O		50	2.82
P		13	18.75
Carcass backfat	3847		
1		3	11.11
2		336	4.66
3		52	1.71
Cold carcass weight (kg)	3862		
<222.5		44	4.25
223-245		17	1.67
245.5-269.5		18	1.96
≥270		15	1.69

[1]n= number of animals corresponding to the criterion

Given that these authors did not really measured meat pH, these results need to be considered with some caution. In agreement, King et al. (2006) reported that cattle with more excitable temperaments had more extensive responses to a stimulated stress challenge and higher basal concentration of glucocorticoids, suggesting that stress response mechanisms are much more active in excitable animals than in their calmer counterparts, being the meat quality affected.

In contrast to expected, the incidence of mountings behaviour in males was 22% greater ($P < 0.001$) in lower stocking densities at slaughterhouse (≤ 0.26 to 0.30 animals per m^2), than in a greater stocking densities (Table 11). In agreement, ultimate pH ≥ 5.8 was greater (mainly in males) when stocking density at slaughterhouse decreased close to 0.26 animals per m^2 (Table 8). These results suggest that decreasing animals per m^2 the rate of agonistic interactions between them increased inducing probably glycogen depletion. The proportion of animals per pen resting was 41.9 ± 1.59%, drinking 3.8 ± 0.37%, and ruminating 7.7 ± 0.53%. Resting and drinking behaviours were not affected by pre-slaughter factors studied, and the interactions between them were not significant. However, ruminating behaviour was greater ($P < 0.001$) in females than in males (IRR = 1.68; data not shown), and slightly decreased ($P < 0.05$) as waiting time at the slaughterhouse increased above 12 h.

Table 8. Results from the logistic model of ultimate meat pH

Item	OR[1]	SEM(OR)	*P*-value[2]
Animal			
Gender			0.08
Male	2.150	0.419	
Female	1	.	
Slaughterhouse			
Waiting time at slaughterhouse (h)			<0.001
≤8.16	1	.	
8.17-11.86	1.117	0.134	
11.87-15.80	1.128	0.135	
≥15.81	2.195	0.240	
Carcass characteristics			
Backfat carcass classification (From 1 to 3)			<0.001
1	1	.	
2	0.371	0.125	
3	0.177	0.058	
Interactions between main factors			
Gender x backfat carcass classification			0.08
1	0.201	6.987	
2	5.88	0.1227	
3	2.08	0.043	
Gender x stocking density at slaughterhouse (animals per m^2)			< 0.01
≤0.26 animals per m^2	1.35	0.142	
0.27-0.30 animals per m^2	2.09	0.089	
0.31-0.37 animals per m^2	1.87	0.760	
≥0.38 animals per m^2	2.79	0.053	

[1] OR correspond to the analyses of the singles factors or to their interactions, being female the reference in the interactions.

[2] *P*-values correspond to the whole model which included gender, the waiting time at slaughterhouse, the backfat carcass classification, the interaction between gender and stocking density at slaughterhouse, and the interaction between gender and backfat carcass classification, as fixed effects, and truck, as a random effect.

Table 9. Results from the logistic model of extreme carcass bruises

Item	OR[1]	SEM(OR)	*P* value[2]
Animal			
Gender class			< 0.001
Male	1.44	0.058	
Female	1	.	
Transport			
No more than one farm in the truck			< 0.01
Yes	1.54	0.0052	
No	1	.	
Stocking density in the truck (animal per m^2)			< 0.01
< 1.29	1.54	0.004	
≥1.30	1		
Slaughterhouse			
Waiting time at slaughterhouse (h)			<0.001
≤8.16	1	.	
8.17-11.86	1.71	0.154	
11.87-15.80	0.68	0.125	
≥15.81	0.68	0.120	
Carcass characteristics			
Hot carcass weight (kg)			< 0.001
≤222.5	1		
223-245	0.39	0.098	
245.5-269.5	0.46	0.100	
≥270	0.39	0.097	

[1] OR correspond to the analyses of the singles factors or to their interactions.

[2] *P*-values correspond to the whole model which included gender, the mixing unacquainted animals from the same origin in the truck, the stocking animal density in the truck, the transport distance, the waiting time at slaughterhouse, the stocking density at the slaughterhouse and the hot carcass weight, as fixed effects, and truck, as a random effect.

Table 10. Effect of waiting time at slaughterhouse on males and females behaviour

	Treatment							
Items	Males				Females			
Waiting time, h	≤ 8.16	8.17-11.86	11.87-15.80	≥15.81	≤ 8.16	8.17-11.86	11.87-15.80	≥15.81
Mountings x pen/5 min	0.28	0.18	0.93	0.5	0.08	0.09	0.12	0.12
Animals ruminating, %	8.9	6.2	5.2	5.2	10.1	11.9	9.9	5.7
Animals resting, %	39.0	41.4	41.9	40.3	32.4	32.4	52.6	55.0

Table 11. Effect of stocking density at slaughterhouse on males and females behaviour.

Items	Treatment							
	Males				Females			
Stocking density, animals/ m^2	≤ 0.26	0.27-0.30	0.31-0.37	≥0.38	≤ 0.26	0.27-0.30	0.31-0.37	≥0.38
Mountings x pen/5 min	0.66	0.81	0.26	0.15	0.10	0.10	0.13	0.06
Animals ruminating, %	4.3	3.6	6.7	10.0	7.5	9.9	11.4	9.3
Animals resting, %	34.2	37.7	39.5	46.8	39.9	38.9	53.75	47.3

4. IMPLICATIONS

The incidence of meat with meat ultimate pH above 5.8 and 6.0 were 13.89% and 4.02%, respectively, and the incidence of severe carcass bruises was 2.43%, representing a great economic impact in the Spanish meat industries. Although several factors significantly affected ultimate meat pH and carcass bruises incidence, the variability explained by the models (4.1% and 13% respectively) was too low, to allow the proposal of management, technical, and economic decisions to improve meat quality in the Spanish market.

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CHAPTER IV: BURDIZZO PRE-PUBERTAL CASTRATION EFFECTS ON PERFORMANCE, BEHAVIOUR, CARCASS CHARACTERISITICS AND MEAT QUALITY OF YOUNG HOLSTEIN BULLS FED HIGH-CONCENTRATE DIETS

CHAPTER IV:

ABSTRACT

In this study the effects of *Burdizzo* pre-pubertal castration on performance, behaviour, carcass, and meat quality of Holstein bulls fed high-concentrate diets were evaluated. Two hundred bulls (8.0 ± 0.42 months) were randomly assigned to control or *Burdizzo* castration. After 121 days, ADG, BW and HCW were greater in intact animals than in castrated animals, as well as, the agonistic and sexual behaviour. However, carcass fatness and intramuscular fat percentage were greater in castrated animals than in intact animals. Additionally, intact animals showed lower L*, a*, and b* than castrated. The Warner-Bratzler Shear Force in intact and castrated animals decreased ($P < 0.01$) from 0 to 7 days of aging time. Additionally, at day 0 of ageing period, meat from castrated animals showed similar shear force values to meat from intact bulls after 7 days of ageing period. However, 23% of castrated animals did not have a complete testicular atrophy, suggesting that the method of castration was not completely effective.

Keywords: Beef; Castration; Performance; Behaviour; Meat Quality

CHAPTER IV:

1. INTRODUCTION

In Spain, meat quality from Holstein bulls (66.8% of total bulls produced) needs to be improved since 62.7% of carcass conformation are classed as “O”, and 32.5% of carcasses backfat are classed as “2” using the EU Regulation (Mach et al., 2008a). Therefore, castration has been proposed as a means of enhancing meat quality. Castration of bulls increases carcass backfat (Field, 1971; Knight et al., 1999; Knight et al., 1999), intramuscular fat content (Knight et al., 1999; Purchas et al., 2002), tenderness (Morgan et al., 1993; Purchas et al., 2002), and reduces the incidence of high ultimate meat pH (Knight et al. 1999; Morgan et al., 1993). Additionally, castration of bulls reduces aggressive and sexual behaviours, improves animal handling, and reduces carcass bruises (Jago et al., 1997; Katz, 2007; Price et al., 2003). The differences in performance between intact bulls and castrates are mainly manifested after puberty as a consequence of a greater production of anabolic hormones by the testes (Adams et al., 1996), which is attained at an average age of 6-9 months (Lunstra et al., 1978). Therefore, Knight et al. (1999) have proposed post-pubertal castration of bulls (13 months of age), as a means to maintain the performance advantages of intact males (greater growth rate and feed efficiency) until 13 months, and the benefits of castration on meat quality characteristics afterwards. However, in Spain, Holstein bulls are slaughtered with an average age of 12 months, and there is a lack of information concerning the effects of pre-pubertal castration (7 - 9 months of age) on animal performance and carcass and meat quality. Therefore, the aim of this study was to determine the effects of pre-pubertal castration using the *Burdizzo* technique on performance, behaviour, carcass characteristics, and meat quality of young Holstein bulls fed high-concentrate diets.

2. MATERIALS AND METHODS

2.1. Animals, Housing, and Treatments

Two hundred Holstein bulls (7.0 ± 0.42 months) were used in a complete randomized design. Bulls were weighed and distributed in two identical buildings with 4 identical pens (25 ± 1 animals per pen). Each pen was randomly assigned to 1 of the following treatments (n=100 bulls/treatment): 1) intact bulls as a control; 2) *Burdizzo* castration. After an adaptation period of 30 d, bulls (8.0 ± 0.42 months) were weighed again and the corresponding animals were castrated (0 day). The average initial BW was 341 ± 5.5 kg and 331 ± 5.5 kg for intact bulls and castrated animals, respectively. The initial BW was not balanced within treatments because mixing young bulls at the beginning of the finishing period could induce social stress (Mounier, Veissier, & Boissy, 2005). The castrated animals were castrated with a standard *Burdizzo* instrument (La

Burdizzo, Corso Sebastopoli 187, Turin, Italy) as described by Fisher et al. (1996). Ten min before castration, 1 mL of local anaesthesia (Lidocaine hydrochloride 2%; Xilocaina Ovejero, Laboratorios Ovejero, Spain) was injected into each testicular cord. Also 25 mL of none-steroidal anti-inflammatory (Flunixin Meglumine; Flunixin Inyectable Norbrook; Laboratorios Karizoo S. A., Spain) were injected intramuscularly. The anti-inflammatory treatment was repeated 2 days after castration. Bulls were fed concentrate (Table 1) and barley straw (3.5% CP, 1.6% EE, 70.9% NDF, and 6.1% ash; DM basis) in separate feeders, both *ad libitum*, for 121 days. Within each building, pens that belonged to the same treatment shared concentrate silo. Bulls were housed at a commercial farm (Montgai, Spain) and managed following the principles and specific guidelines of IRTA Animal Care Committee.

Table 1. Ingredients and chemical composition of the concentrate

	% of DM
Ingredients	
Corn grain meal	45.6
Soybean hulls	16.0
Barley grain	12.0
Beet pulp	8.0
Soybean meal	4.7
Linseed premix[1]	4.5
Wheat middlings	3.8
Extrused whole soybean seed	3.0
Calcium carbonate	1.1
Palm oil	0.8
Salt	0.3
Vitamin premix[2]	0.2
Analyzed nutrient content	
ME, Mcal/kg	2.84
CP, % of DM	14.8
Ether extract, % DM	5.7
NDF, % of DM	25.5
Ash, % of DM	4.4

[1] Every kilogram contained 60% of linseed, 40% of barley grain, 0.30% of calcium carbonate, with a chemical composition of 17% CP, 24% EE, and 7.5% ash.

[2] Every kilogram contained 9,000 IU Vit A; 2,000 IU Vit D3; and 50,000 IU Vit E, 30,000 mg of Magnesium Oxide, 60,000 mg of Cooper Sulfate Pentahydrate, 60,000 mg of Zinc Oxide, 1,000 mg of Cobalt, 1,000 of Iodine and 200 mg of Selenium.

2.2. Measurements and Sample Collection

The amount of concentrate and straw offered were recorded daily from 0 to 121 d. After 121 days of experiment, the amount of concentrate that remained in the silos and feeders was weighed in order to estimate the total amount of concentrate and straw consumptions. Animal BW was recorded on day -30, 0, 30, 60, 90, and 121 of experiment. In addition, animal behaviour was registered on day -2, 2, 30, 60, 90 and 121 of experiment from 07:30 to 10:00 am. Whereas animal social behaviours (agonistic interactions, nonagonistic interactions, and sexual interactions) were scored using a 2 continuous behaviour sampling of 15 min each day, the general activities of animals were scored using 6 scan sampling of 10 s at 5 min interval (Mounier et al., 2005). Agonistic interactions registered were fighting (when bulls pushed vigorously head against head), butting (when one bull pushed vigorously its head against any part of another bull's body), displacement (when one bull shoved itself between two other animals or between an animal and a wall or any equipment), chasing (when one bull made another animal flee by following fast or running behind it), and chasing-up (when one bull used forceful physical contact against a resting animal which made the receiver rise). Nonagonistic interactions included social licking (when one bull touched with its tongue any part of another animal's body), self-licking, and horning (same as a fighting but without violent contact). Sexual interactions included: flehmen, attempted mounts (head on the back of another animal), and completed mounts. The general activity of animals were resting, standing, eating concentrate (number of animals located in the feeders with their heads put through the feeding rack), and ruminating.

At day 121 of study, 25 intact animals (535 ± 2.4 kg) and 25 castrated animals (515 ± 2.4 kg) were randomly selected and transported to a commercial slaughterhouse (Mercabarna, Barcelona, Spain). Truck stocking density was 0.8 ± 0.23 animals/m^2, and transport distance was less than 150 km. This stocking density is in accordance with the density recommended by European Community directives (64/432/EC and 93/119/EC) for 500 kg cattle. Animals from different treatment and pen were not mixed in the truck. At the slaughterhouse, animals were housed in 10 different pens (5 pens per treatment), with 0.25 animals/m^2, for approximately 4 h before slaughter. Within the treatment, animals from different pens were not mixed. Animals were stunned using a captive-bolt pistol and dressed according to commercial practices. The testes weight was also recorded at slaughter. During exsanguination in the slaughterhouse, blood samples (10 mL) were taken. Blood samples were centrifuged at 1500 x *g* at 4ºC for 15 min, and serum was stored at -20ºC until subsequent analysis for serum testosterone concentration. The HCW was recorded, and carcass fatness and conformation were graded according to the EU classification system into 1.2.3.4.5 (EU Regulation nº 1208/81) and into (S)EUROP categories (EU Regulation nº 1208/81, 1026/91), respectively. The conformation class designated by the letter "E" (Excellent) describes carcasses with all profiles convex to super-convex, and with exceptional muscle development, whereas the conformation classified as "U" (Very good) describes carcasses with profiles on the whole convex, and with very good muscle development. The carcasses

classified as “R” (Good) present profiles on the whole straight, and good muscle development. Carcasses classified as “O” (Fair) present profiles straight to concave, and with average muscle development, whilst carcasses classified as “P” (Poor) present all profiles concave to very concave with poor muscle development. In addition, the degree of backfat describes the amount of fat on the outside of the carcass and in the thoracic cavity. While the class of backfat that classifies as 1 (Low) describes none to low fat cover, the class of backfat classified as 5 (Very High) describes an entire carcass covered with fat and with heavy fat deposits in the thoracic cavity. Dressing percentage was calculated from HCW. After 24 h of carcass chilling, a sample of *M. longissimus* (LM) between the 6th and 9th ribs was removed from each carcass.

2.3. Chemical Analyses

Feed samples were analyzed for DM (24 h at 103ºC), ash (4 h at 550ºC), CP by the Kjeldahl method (AOAC, 1995), NDF according to Van Soest et al. (1991) using sodium sulphite and alpha-amylase, and fat by Soxhlet (AOAC, 1995).

Serum concentration of testosterone was determined using a solid phase radioimmunoassay (Kit Coat-A-Count Total Testosterone, Diagnostic Products Corporation, Los Angeles, USA).

Ultimate meat pH was measured using a Crison portable meter equipped with a xerolyt electrode inserted in the LM at the 6th rib at 24 h *post-mortem* and at 5 ± 1ºC. The LM area was measured on a digital image obtained from the exposed surface muscle area between the 5th and 6th thoracic ribs, and calculated using an imaging software (Pomar et al., 2001). Instrumental colour measurements were recorded at 24 h *post-mortem* for L* (lightness), a* (redness), and b* (yellowness) on the exposed cut surface of the LM between the 6th and 7th ribs after 30 min of bloom time, using a Minolta Chromameter (CR-400, Minolta Inc., Osaka, Japan) in the CIELAB space (CIE, 1976). Seven steaks (2.0 cm) were cut between the 7th and 9th ribs of the LM and vacuum packaged individually. One sample was stored at -20ºC until determination of intramuscular fat (IMF) percentage. Six steaks were stored in a cooler at 4°C and frozen after 0, 7 and 14 days of ageing for subsequent sensory analysis and Warner-Bratzler shear force (WBSF) evaluation. Intramuscular fat percentage of LM was predicted using Near Infrared Transmission (NIT) spectroscopy spectrum in the region 850-1050 nm (Infratec 1265 Meat Analyzer, Tecator AB, Uppsala, Sweden), calibrated for pork from 0 to 8% of fat range and validated for beef (Mach et al., 2008b). A sample of 250 g of LM was homogenized prior to NIT analysis using a food processor (Robot coupe Blixer3, Montceau Les Mines, France). Intramuscular fat content was expressed as g of fat/100 g of muscle. Meat samples obtained for WBSF measurements were wrapped in aluminium foil and cooked to an internal temperature of 71ºC in a convection oven pre-heated to 200ºC. Sample internal temperature was monitored with a data logger and a thermocouple probe inserted horizontally at the steak midpoint. The maximum shear force was determined from 5

replicates (1 cm^2 cross-section, 3-cm long) with fiber direction parallel to the longest dimension of the strip and perpendicular to the direction of the blade (Honikel, 1998), using a texture analyzer (Alliance RT/5 MTS Systems Corp. Eden Prairie, Minneapolis, USA) equipped with a Warner-Bratzler blade. For sensory attributes evaluation, thawing and cooking were accomplished using the same protocol described previously for WBSF determination. After cooking, each sample was cut into 8 subsamples. Each subsample was immediately wrapped in aluminium foil, codified and kept in a heater to maintain a constant temperature of 60ºC until to panelist assessment (Serra et al., 2008). Panelists evaluated the cooked subsamples in individual booths provided with red light. No more that 2 sessions (6 samples per session) were conducted per day. The subsamples were tasted in different order in each session to avoid carry-over effects (MacFie, and Thompson, 1988). Before tasting each subsample, panelists were required to eat some unsalted toasted bread and then rinse their mouths with water. Panelists were required to rate each subsample for beef flavour, tenderness and juiciness. Each attribute was rated on a non-structured 10-point scale, with score 0 equivalent to no attribute intensity and score 10 equivalent to the highest intensity of the attribute.

2.4. Statistical Analyses

Performance data were analyzed using mixed-effects ANOVA with repeated measures (SAS Inst. Inc., Cary, NC). The model included initial BW as a covariate, treatment, time, and the interaction between them, as fixed effects, and the animal, pen, and building, as random effects. Time was considered a repeated factor, and for each analyzed variable, animal nested within building, pen, and treatment was subjected to compound symmetry variance-covariance structures. Concentrate and straw consumptions were analyzed as described for performance but without the time effect. To analyze behaviour data, pen was considered the experimental unit. The social behaviour data were analyzed using a Poisson regression. The model included treatment, time, and their interactions, as fixed effects, and pen and building as random effect. The number of animals in each pen was included as a weight variable. The Incidence Relative Ratio (IRR) was calculated. The general activities of animals were averaged by day for each pen, transformed into natural log to achieve a normal distribution, an analyzed using mixed-effects ANOVA. The model included the treatment, time, and their interactions, as fixed effects, and the pen nested within building as random effects. The serum concentration of testosterone was also transformed to a natural log scale. The model included final BW as covariables, treatment as fixed effect, and the animal, pen, and building as random effect. Testes weight, and carcass and meat quality characteristics were analyzed as described for performance but without the time effect, and final BW was used as a covariate instead of initial BW. A Chi-square-test was conducted to test the effects of treatment on categorical variables (carcass classification data). Sensorial data were analyzed using mixed-effects ANOVA. The statistical model considered the treatment, ageing time, and their interactions as fixed effects, and the animal nested within panelist and session as a random effect.

3. RESULTS AND DISCUSSION

Three castrated animals and 2 intact bulls were removed from the study due to health problems unrelated to the treatments.

3.1. Intake, and Animal Performance

Daily concentrate intake (7.6 ± 0.08 Kg of DM/d) and total DMI (8.4 ± 0.04 kg of DM/d) were not affected by castration (Table 2).

Table 2. Intake and performance of Holstein bulls fed high-concentrate diets after pre-pubertal castration using the *Burdizzo* technique

	Treatment[1]			
Item	CTR	BURD	SEM	*P*- value[2]
Initial BW, kg	341	331	5.5	0.05
Final BW, kg	535	515	2.4	0.01
ADG, kg/d	1.62	1.53	0.05	0.01
Concentrate DMI, kg /d	7.73	7.43	0.08	0.001
Straw DMI, kg/d	0.72	0.93	0.05	0.74
Total DMI, kg/d	8.45	8.36	0.04	0.83
G: F ratio, kg/kg	0.21	0.20	0.002	0.11

[1]CTR: Control; BURD: *Burdizzo* castration

[2]Effect of castration

To our knowledge, there are no studies that evaluated changes in DMI of young bulls above the 35 days after the castration. However, supporting the present results, some studies conducted with Holstein bulls castrated at 5.5 months of age (Fisher et al., 1996; Pang et al., 2006) have reported no negative effects on feed intake during the first 35 days after the castration. Animal initial BW was greater ($P < 0.05$) in intact bulls (341 ± 5.5 kg) than in castrated animals (331 ± 5.5 kg), and the BW difference between castrated and intact animals increased linearly ($P < 0.001$) from day 0 to 121 days of study (from 10 ± 0.4 to 20 ± 0.4 kg). Furthermore, the ADG was greater ($P < 0.01$) in intact bulls (1.6 ± 0.05 kg/d) than in castrated animals (1.5 ± 0.05 kg/d). In agreement with these results, previous studies (Fisher et al., 1996; 1997; Early and Crowe, 2002) have reported that animals castrated at 5 months of age presented lower ADG after castration than intact bulls. Castration did not affect feed efficiency (0.20 ± 0.002 kg/d), probably because the concentrate intake of castrated animals was numerically lower than that for intact bulls (Table 2). The lesser growth rate of castrates compared with intact bulls seems to be due to a reduction of natural anabolic hormones production by the testes (Adams et al., 1996; Frietsche and Steinhart, 1998; Knight et al., 1999), and immediate tissue trauma, inflammation, and associated acute stress (Chase et al., 1995; Fisher et al., 1996; 1997; Early and Crowe, 2002; Ting et al., 2003; Pang et al., 2006).

3.2. Animal Behaviour

The proportion of animals per pen resting (16.3 ± 1.31%), drinking (1.2 ± 0.17%), eating concentrate (9.7 ± 0.50%), eating straw (8.4 ± 0.56 %), and ruminating (6.2 ± 0.52%) were not affected by castration, and the interaction between time and treatment was not significant. To our knowledge, there are no studies that reported the general activities of animals for long-term period after castration. However, Ting et al. (2003) reported that Holstein bulls surgically castrated at 11 months of age, remained in greater proportion of standing and lower resting during the first 6 h after castration, compared with control. In contrast, Robertson et al. (1994) did not report any altered postures or behaviour during the first 2 h after the castration of calves castrated with *Burdizzo* at 6, 21 or 42 days of age. Related to agonistic interactions, the incidence of animal fighting and displacements per pen was greater ($P < 0.01$) in intact than in castrated animals (IRR = 2.86 and IRR= 1.19, respectively). Average incidence of animal fighting was 0.3 ± 0.11 and 0.1 ± 0.05 times/5 min in intact and castrated animals, respectively, whereas the average incidence of animal displacements per pen was 2.3 ± 0.39 and 1.9 ± 0.32 times/5 min in intact and castrated animals, respectively. No differences in butting, chasing and chasing-up behaviours were found between treatments. Additionally, the nonagonistic interactions were not affected by treatment, being the average incidence per pen of animal horning, self-licking and social licking 4.8 ± 0.33, 3.9 ± 0.49, and 5.2 ± 0.40 times/5 min, respectively. As expected, the frequency of mounts in castrated animals acutely decreased ($P < 0.01$) from 60 to 121 days after the castration (from 2.5 to 0.3 times/5 min per pen), whereas in intact animals remained constant (3.0 ± 0.56 times/5 min per pen). In addition, attempted mounts also acutely decreased ($P < 0.01$) from 60 to 121 days after the castration (from 2.3 to 0.1 times/5 min per pen), remaining constant in intact bulls (2.9 ± 0.46 times/5 min per pen). Furthermore, flehmen response in castrated animals decreased ($P < 0.01$) from 30 to 121 days (from 1.8 to 0.4 times/5 min per pen), remaining constant in intact animals (2.4 ± 0.33 times/5 min per pen). These results indicate that *Burdizzo* castration of pre-pubertal bulls resulted in a reduction of agonistic and sexual behaviours from 60 to 121 days after castration. Although there are no studies that report the agonistic, nonagonistic and sexual behaviour of pre-pubertal bulls castrated by *Burdizzo* method, supporting the present results, Katz (2007) have reported a decrease of sexual behaviour incidence following castration, and Huxsoll et al. (1998) also have reported that active immunization against GnRH not only reduced the serum concentration of testosterone, but also decreased the magnitude of aggressive behavioural traits in intact bulls compared with castrates.

3.3. Testes Characteristics

As expected, the average testes weight collected at slaughter was greater ($P < 0.001$) in intact bulls (520.7 ± 29.96 g) than in castrated animals (169.7 ± 27.32 g). Therefore, visual assessment of testes size at the slaughterhouse would be a fast and practical method to predict if animals are well castrated. Additionally, serum concentration of testosterone at slaughter was

greater ($P < 0.05$) in intact animals (3.7 ± 0.68 ng/mL) than in castrated animals (1.8 ± 0.68 ng/mL). In agreement, Price et al. (2003) analyzing animals of 16 months of age reported that intact bulls presented greater serum concentration of testosterone (2.9 ± 0.41 ng/mL) than animals surgically castrated at 4 months of age (0.1 ± 0.01 ng/mL). Serum concentration of testosterone was not completely reduced to zero in castrated animals. These results are probably because about 23% of castrated animals did not have a complete testicular atrophy (testes size was not completely reduced), suggesting that the tension applied to the *Burdizzo* clamp when animals were castrated was not sufficient to eliminate the blood supply to the testes and scrotum. Therefore, the *Burdizzo* castration method might not be 100% affective to use in pre-pubertal cattle.

3.4. Carcass and Meat Quality Characteristics

Carcass and meat quality characteristics are presented in Table 3. Final BW achieved 121 d after castration, was greater ($P < 0.01$) in intact animals (535 ± 2.4 kg) than in castrated animals (515 ± 2.4 kg; Table 2). In addition, HCW was greater ($P < 0.05$) in intact animals (283 ± 4.2 kg) than in castrated animals (267 ± 4.2 kg), without differences in dressing percentage. Supporting the present results, Knight et al. (1999) found that the final BW and HCW of castrated animals at 17 months of age and slaughtered 5.4 months later were lower than that of intact bulls. Castration did not affect carcass conformation (89% classified as “O”, Table 3). However, carcass backfat was greater ($P < 0.05$) in castrated animals (94% classified as “3”) than in intact bulls (67% classified as “3” and 33% classified as “2”), as it has been previously reported by Knight et al. (1999) and Morgan et al. (1993). As expected, the IMF percentage was greater ($P < 0.001$) in castrated animals (4.1 ± 0.23%) compared with intact bulls (2.6 ± 0.23%). The LT area was lower ($P < 0.01$) in castrated animals (36.0 ± 1.90 cm^2) compared with intact bulls (44.2 ± 1.90 cm^2). Similar findings have been supported by other authors (Knight et al., 1999; Morgan et al., 1993), as a response to the testicular function suppression. The LM pH (5.5 ± 0.01) was not affected by treatment. However, intact bulls presented a darker LM than castrated animals at 24 h *post-mortem* as indicated by the lower L* value (34.7 ± 0.29 for intact bulls and 35.3 ± 0.29 for castrated animals, respectively; $P < 0.05$). The LM of intact bulls tended to have a lower a* value (14.1 ± 0.75 for intact and 15.5 ± 0.75 for castrated animals, respectively; $P = 0.07$), and showed lower b* value (6.4 ± 0.55 for intact and 7.2 ± 0.55 for castrated animals, respectively; $P < 0.01$) compared with castrated. It is often reported that intact males compared with castrates produce darker meat (Monin and Ouali, 1991). Because similar levels of myoglobin were found in bulls and steers (Field, 1971), the darker colour of bull meat was ascribed to its higher pH, as a result of their more excitable temperament and therefore of higher rates of ante-mortem glycolysis (Monin, 1990). However, in the present study no differences in ultimate meat pH were reported between intact and castrated animals. Thus, it was probably because of castrated carcasses had more external fat cover and intramuscular fat content than intact bulls (Table 3).

Table 3. Carcass and meat quality of LM from Holstein bulls fed high-concentrate diet after pre-pubertal castration using the *Burdizzo* technique

Item	Treatment[1]		SEM	*P*-value[2]
	CTR	BURD		
Carcass				
Hot weight, kg	283.5	267.0	4.23	0.04
Cold weight, kg	278.0	263.5	4.54	0.04
Dressing percentage, %	52.9	51.8	0.12	0.40
LM				
Ph	5.53	5.51	0.012	0.75
Area, cm^2	44.2	36.0	1.90	0.01
Instrumental colour [3]				
*L**	34.7	35.3	0.29	0.02
a*	14.1	15.5	0.75	0.07
b*	6.4	7.2	0.55	0.01
Intramuscular fat content, %	2.61	4.10	0.233	0.001
WBSF, kg[4]				
Day 0	5.61	4.10	0.202	0.01
Day 7	4.22	3.01	0.203	0.01
Day 14	3.93	3.14	0.205	0.01

[1]CTR: Control; BURD: *Burdizzo* castration

[2]Effect of castration

[3]Colour: L*=lightness, a*=redness, and b*= yellowness

[4]Warner-Bratzler Shear Force

Tenderness has been identified as the main factor determining the consumer-eating satisfaction of beef (Jeleníková et al., 2008). The WFSF in intact and castrated animals decreased ($P < 0.01$) from 0 to 7 days of ageing time (Table 3). Additionally, meat from castrated animals at day 0 of the ageing period showed similar shear force values to meat from intact bulls after 7 days of ageing. Although meat ageing is recommended for the development of the organoleptic qualities of meat, present results suggest that meat from castrated carcasses does not need an extend ageing to achieve an acceptable degree of tenderness. It is also interesting to note that, in the present study, *Burdizzo* castration failed in 23% of the cases. This suggests that at 100% of castration efficiency, there would be an even greater difference in muscle tenderness between castrated and intact animals. Since animals were slaughtered 12 months of age, connective tissue content/composition may not have had a great impact on tenderness. Therefore, differences in WBSF between castrated and intact bulls may be due to other factors such as sarcomere length or fibre type and diameter, protein profile and its proteolysis. In agreement with shear force values, sensory attributes ratings of tenderness indicated that as *post-mortem* ageing period increased, tenderness increased ($P < 0.01$), being greater for castrated animals than for intact animals (Table 4). In addition, the meat from intact bulls tended ($P = 0.11$) to present less juiciness than castrated animals, which may be attributable partially to a 20% lower intramuscular fat percentage in intact bulls than in castrated animals, and partially to lower tenderness of meat. As greater the meat

tenderness, the more quickly the juices are released by chewing and the more juicy the meat appears (Cross, 1988). Supporting present results, Purchas et al. (2002) reported that meat from bulls castrated at 2 months of age and slaughtered at 16 to 18 months of age was more tender than meat from intact bulls. The authors associated this greater tenderness of meat from castrated animals with a slightly lower ultimate pH, greater myofibrillar fragmentation indexes, more intramuscular fat, greater cooking losses, and possibly, a lesser contribution of connective tissue components. In agreement, Morgan et al. (1993) reported greater myofibril fragmentation index values (indicating less proteolysis) occurred in meat from animals castrated at 1 week of age compared with meat of intact bulls. Flavour was not affected by treatment. It is important to notice that the average carcass and meat quality could be further improved if the *Burdizzo* castration method would be 100% effective in pre-pubertal bulls.

Table 4. Sensory quality of LM from Holstein bulls fed high-concentrate diet after pre-pubertal castration using the *Burdizzo* technique

	Treatment[1]			
Attribute[3]	CTR	BURD	SEM	*P*-value[2]
Tenderness				
Day 0	3.89	5.64	0.38	0.001
Day 7	5.53	7.36	0.38	0.001
Day 14	6.20	7.58	0.38	0.001
Juiciness	4.78	5.26	0.26	0.11
Beef flavour	6.22	6.31	0.28	0.61

[1]CTR: Control; BURD: *Burdizzo* castration

[2]CAS= effect of castration; AT= ageing time

[3] 10 cm unstructured line scale

4. IMPLICATIONS

Pre-pubertal castration of Holstein bulls at 8 months of age using the *Burdizzo* technique reduced ADG, final BW, and HCW compared with intact bulls. However, castration of bulls reduced fighting, displacements, and sexual behaviour. Furthermore, castration improved carcass and meat quality, increasing carcass fatness, intramuscular fat content, tenderness, and colour lightness and redness. However, above 23% of castrated animals did not have a complete testicular atrophy, suggesting that the *Burdizzo* method might not be 100% effective in pre-pubertal cattle.

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CHAPTER V: INCREASING THE AMOUNT OF OMEGA-3 FATTY ACID OF MEAT FROM INTENSIVELY FED YOUNG HOLSTEIN BULLS THROUGH NUTRITION

CHAPTER V:

ABSTRACT

Fifty-four bulls were blocked by initial BW (301 ± 7.4 kg) and randomly assigned to 6 treatments following a 3 x 2 factorial design with 3 concentrate lipid levels (5, 8, and 11% of DM) and 2 lipid sources (whole canola seed and whole linseed) with the objective of evaluating the possibility of increasing the content of omega-3 (n-3) fatty acids in meat. Concentrates (mostly corn meal) were isonitrogenous and isocaloric. Concentrate and straw were both fed *ad libitum.* Animal BW was recorded every 2 week, and feed consumption weekly. Ruminal pH and VFA concentrations were determined monthly. Bulls were transported to the slaughterhouse when they achieved the target slaughter weight of 440 kg (after 105 ± 4 d of fattening). After slaughter, a sample of M. *longissimus* (LM) from the 6th to the 8th ribs was dissected and analyzed for intramuscular fat content and fatty acid profile. Dietary lipid source did not affect overall animal performance, rumen fermentation, or carcass and meat quality. Rumen pH was > 6.0 despite bulls consuming large amounts of concentrate. In bulls fed linseed, the percentage of n-3 fatty acids in LM increased linearly with lipid level, whereas in bulls fed canola seed it remained constant. The ratio of n-6:n-3 fatty acids was lower ($P < 0.01$) in the LM of bulls fed linseed (10.0) than in those fed canola seed (26.0). The content of *cis*-9, *trans*-11-CLA in the LM tended ($P = 0.06$) to be greater in the bulls fed linseed than in those fed canola seed (62.9 and 49.2 mg/kg of LM, respectively). Concentration of n-3 fatty acids in meat of bulls fed high-concentrate diets can be enhanced by whole linseed supplementation without affecting animal performance, ruminal fermentation, or carcass quality.

Key words: *Beef, CLA, Omega-3, Linseed, and Rumen*

CHAPTER V:

1. INTRODUCTION

Omega-3 (n-3) fatty acids (FA), especially 20:5n-3 (EPA), and 22:6n-3 (DHA), have been reported to exert beneficial effects on cardiovascular health (Simopoulus, 1999; Krauss et al., 2000; Lee and Lip, 2003) and play important biological functions, particularly in inflammation, brain development, sight, and immune function (Connor, 2000). The American Heart Association (Krauss et al., 2000) recommended a reduction in the consumption of saturated fatty acids (SFA) and an increase in the consumption of unsaturated FA, mainly n-3, to obtain a ratio of omega-6 (n-6) to n-3 FA of 4.0 or less. The ratio of n-6:n-3 FA can be improved by decreasing n-6 FA consumption, increasing n-3 FA consumption, or both. More recently, Wijendran and Hayes (2004) have described the importance of providing a ratio of n-6: n-3 FA close to 6.0 in human diets, but have emphasized that the first consideration when contemplating long term consumptions of fatty acids should be the absolute amounts of n-6 and n-3 consumed rather than their ratio. In that sense Wijendran and Hayes (2004) recommended 1.7 g/d of *cis*-9, *cis*-12, *cis*-15-18:3 (ALA) based on the reduction of platelet aggregation observed in hyperlipidemic subjects supplemented with this amount of ALA (Freese et al. 1994).

To enrich beef with n-3 FA, the dietary supply of n-3 FA must escape rumen biohydrogenation (which converts unsaturated FA to SFA) before it can be absorbed in the small intestine and deposited in meat. One strategy to avoid rumen biohydrogenation is to feed whole oilseeds, because the seed coat prevents the access of rumen microorganisms to the unsaturated FA (Aldrich et al., 1997). The n-3 FA content of muscle has been increased in late maturing breeds of cattle by feeding forage-based diets supplemented with oils or oilseeds rich in ALA, EPA, or DHA (Choi et al., 2000; Scollan et al., 2001a; Raes et al., 2004a). However, there are no studies conducted with cattle of early maturing breeds and less than 12 mo of age. The objective of this study was to assess the possibility of enriching the concentration of n-3 FA, especially ALA, and to improve the ratio n-6:n-3 FA in meat from young Holstein bulls fed high-concentrate diets using whole linseed.

2. MATERIALS AND METHODS

2.1. Animals, Housing, and Treatments

Fifty-four Holstein bulls were blocked into 3 BW groups (274, 295, and 329 kg) and randomly assigned to 1 of 6 dietary treatments following a 3 x 2 factorial design. The 6 treatments consisted of 3 concentrate lipid levels: 5, 8, and 11% of DM, and 2 lipid sources: whole canola

seed and whole linseed. Whole linseed was chosen because it is an oilseed rich in n-3 FA (54.2% ALA) and its seed coat might protect PUFA from rumen biohydrogenation and increase passage of PUFA to the duodenum (Scollan et al., 2001b). Whole canola seed was chosen as a negative control because it is also a seed coat-protected oilseed rich in PUFA but poor in n-3 FA (10.6% ALA). All concentrate ingredients were ground with the exception of the oilseeds that were included as whole seeds. The 6 concentrates were isonitrogenous and isocaloric (Table 1), but differed in FA profile, mainly due to differences in 16:0, 18:0, *cis*-9-18:1, *cis*-9, *cis*-12-18:2 (LA), and ALA (Table 2).

Table 1. Ingredient and chemical composition of the concentrates

	Concentrate lipid level, % of DM					
		Low (5%)	Medium (8%)		High (11%)	
	Concentrate lipid source					
Item	Canola seed	Linseed	Canola seed	Linseed	Canola seed	Linseed
Ingredient	% of DM					
Corn grain meal	77.0	77.0	58.0	56.0	41.4	44.1
Whole linseed	-	3.6	-	11.2	-	18.0
Whole canola seed	2.9	-	10.0	-	16.1	-
Beet pulp	-	2.3	12.8	14.3	10.0	14.3
Wheat middlings	2.5	-	2.9	2.9	9.0	9.5
Sunflower meal	-	0.4	3.0	0.1	5.6	4.6
Soybean meal	10.4	10.3	5.7	7.5	-	-
Corn gluten feed	2.5	2.1	2.1	2.1	2.1	2.1
Molasses	1.4	1.6	1.0	2.0	1.9	2.0
Oat meal	0.6	-	0.7	-	9.8	1.2
Salt	0.65	0.65	1.22	1.22	1.19	1.22
Dicalcium phosphate	0.43	0.43	0.60	0.61	0.61	0.61
Calcium carbonate	0.40	0.40	0.71	0.71	0.71	0.71
Sodium bicarbonate	0.40	0.40	0.48	0.48	0.70	0.70
Magnesium oxide	0.28	0.29	0.29	0.28	0.29	0.29
Vitamin premix[1]	0.60	0.60	0.60	0.60	0.60	0.60
Nutrients						
ME, Mcal/kg[2]	2.88	2.88	2.88	2.88	2.88	2.88
CP, % of DM	14.3	13.8	14.4	13.9	13.9	14.5
Ether extract, % of DM	5.2	5.4	9.0	8.8	11.6	11.1
NDF, % of DM	11.2	10.8	15.6	16.5	22.7	21.4
Ash, % of DM	5.1	4.9	6.6	7.3	7.6	7.9

[1] Contained 10,000 IU of vitamin A, 2,000 IU of vitamin D3, and 16,667 IU of vitamin E per kg.

[2] Calculated according to INRA (1989).

[3] Nonfibrous carbohydrates = 100 minus the sum of ash, CP, NDF, and fat.

To maintain concentrates isocaloric, the increase in lipid level was counterbalanced by a decrease in non-fibrous carbohydrates (NFC), mainly by reducing corn meal, and an increase in NDF. Bulls were fed concentrate in a trough (0.6 m x 2.65 m) and barley straw (3.5% CP, 1.6% EE,

70.9% NDF, and 6.1% ash on DM basis) in a separate trough (0.6 m x 1.2 m), both for *ad libitum* consumption, until reaching the target slaughter weight of 440 kg. Bulls were housed in outdoor paved and partially covered 13.65-m x 3.85-m pens (3 bulls/pen) at the IRTA experimental station (Prat de Llobregat, Spain). Bulls were managed following the principles and specific guidelines of the IRTA Animal Care Committee.

Table 2. Fatty acid profile of the concentrates

	Concentrate lipid level, % of DM					
	Low (5%)		Medium (8%)		High (11%)	
	Concentrate lipid source					
Fatty acid	Canola seed	Linseed	Canola seed	Linseed	Canola seed	Linseed
	g/100 g total					
8:0	ND[1]	ND	0.05	ND	ND	ND
12:0	ND	ND	0.01	0.01	0.01	0.02
14:0	0.06	0.06	0.06	0.07	0.07	0.07
16:0	11.1	11.2	8.2	9.3	7.4	8.1
16:1, *cis*-7	0.32	0.10	0.16	0.27	0.08	0.30
16:1, *cis*-11	0.19	0.13	0.23	0.11	0.24	0.10
17:0	0.11	0.09	0.11	0.09	0.10	0.08
18:0	2.05	2.77	1.79	3.24	1.66	3.35
18:1, *cis*-9	33.8	23.8	45.4	22.6	49.5	22.1
18:1, *cis*-11	1.49	0.73	2.47	0.77	2.84	0.76
18:2, *cis*-9, *cis*-12	45.8	43.4	33.5	30.4	28.9	25.8
18:3, *cis*-9, *cis*-12, *cis*-15	3.5	16.7	6.1	32.3	7.0	38.4
20:0	0.46	0.36	0.49	0.27	0.52	0.21
20:1, *cis*-11	0.53	0.23	0.84	0.22	1.04	0.22
20:2, *cis*-11, *cis*-14	0.03	0.05	0.07	0.06	0.09	ND
20:3, *cis*-11, *cis*-14, *cis*-17	ND	ND	ND	0.04	ND	0.04
22:0	0.23	0.19	0.29	0.17	0.32	0.17
22:1, *cis*-13	0.05	0.00	0.03	0.04	0.05	0.02
22:2, *cis*-13, *cis*-16 & 23:0	0.05	0.05	0.04	-	0.03	0.04

[1] Not detectable or detected at < 0.01/100 g of total fatty acids.

2.2. Measurements, and Sample Collection

Animal BW was recorded every 2 weeks, and concentrate and straw consumption weekly. Rumenocentesis was performed monthly, 4 h after feeding, during three consecutive days (18 bulls/d) to avoid differences due to sampling time within day.

Rumenocentesis was conducted with a 14-cm 14-gauge needle inserted into the ventral sac of the rumen approximately 15 to 20 cm caudal-ventral to the costocondral junction of the last rib. Rumen liquid pH was measured immediately with a pH meter (model 507 CRISON Instruments

SA, Barcelona, Spain). Based on Jounay (1982), 4 mL of ruminal liquid were mixed with 1 mL of a solution containing 0.2% (wt/wt) mercuric chloride, 2% (wt/wt) orthophosphoric acid, and 4-methylvaleric acid (internal standard) in distilled water, and stored at -20ºC until subsequent VFA analyses.

Bulls were transported to the slaughterhouse when they achieved the target slaughter weight of 440 kg. Immediately following slaughter, HCW was recorded, and carcass backfat and conformation were graded according to the EU classification system into 1.2.3.4.5 (EU Regulation nº 1208/81) and into (S)EUROP categories (EU Regulation nº 1208/81, 1026/91), respectively. The conformation class designated by the letter "E" (Excellent) describes carcasses with all profiles convex to super-convex, and with exceptional muscle development, whereas the conformation classified as "U" (Very good) describes carcasses with profiles on the whole convex, and with very good muscle development. The carcasses classified as "R" (Good) present profiles on the whole straight, and good muscle development. Carcasses classified as "O" (Fair) present profiles straight to concave, and with average muscle development, whilst carcasses classified as "P" (Poor) present all profiles concave to very concave with poor muscle development. In addition, the degree of backfat describes the amount of fat on the outside of the carcass and in the thoracic cavity. While the class of backfat that classifies as 1 (Low) describes none to low fat cover, the class of backfat classified as 5 (Very High) describes an entire carcass covered with fat and with heavy fat deposits in the thoracic cavity. Dressing percentage was calculated from HCW. After 24 h of carcass chilling, carcasses were weighed again, ribbed at 6th and the 8th ribs, and the resulting rib sample was collected. The ultimate meat pH at 24 h of the LM at the 6th rib was measured in the center of the LM using a CRISON portable meter equipped with a xerolyt electrode, and the area of the LM was determined by artificial vision (Pomar et al., 2001). Instrumental colour measurements values of LM were measured with a Minolta (CM-2002, Minolta Co. Ltd., Osaka, Japan) between the 6th and the 7th ribs 24 h *post-mortem* for L* (lightness), a* (redness), and b*(yellowness). Before reading, ribs were kept in dark and cold conditions (4ºC) for 15 min. The LM from the 6th to the 8th ribs was dissected and a 200-g sample was frozen at -20ºC until analysis of fat content and FA composition.

2.3. Chemical Analyses

Feed samples were analyzed for DM (24 h at 103ºC), ash (4 h at 550ºC), CP by the Kjeldahl method (AOAC, 1995), NDF according to Van Soest et al. (1991) using sodium sulphite and alpha-amylase, and fat by Soxhlet with previous acid hydrolysis (AOAC, 1995). The NFC content was calculated as 100% minus the sum of ash, CP, NDF, and fat. Rumen VFA concentration was analyzed with a polyethylene glycol terephtalic acid treated capillary column (25 m x 0.25 mm ID, 0.25 µm film thickness, BP21, SGE, Europe Ltd., Barcelona, Spain) using GLC (Carlo Erba Instruments chromatograph, CE 5300 HT, Milano, Italy) with an initial temperature of 100ºC for 1 min, increased 8ºC/min to 160ºC, then held at 160ºC for 5 min. The injector and flame

ionization detector temperatures were 250ºC and 280ºC, respectively. Carrier gas was He at 30 cm/sec, and the injection was performed by split mode at a ratio of 1:30.

Intramuscular fat percentage of LM was predicted using Near Infrared Transmission (NIT) spectroscopy spectrum in the region 850-1050 nm (Infratec 1265 Meat Analyzer, Tecator AB, Uppsala, Sweden), calibrated for pork from 0 to 8% of fat range. A sample of 250 g of LM was homogenized prior to NIT analysis using a food processor (Robot coupe Blixer3, Montceau Les Mines, France). A subsample of 10 g from homogenized 250 g of LM was used to determine intramuscular fat content by Soxhlet with a previous acid hydrolysis (AOAC, 1995). Intramuscular fat content data were expressed as g of fat/100 g of muscle. Another subsample of 2 g was used to determine FA composition. Fat was extracted as described by Folch et al. (1957); the 2-g subsample was homogenized in 100 mL of a 2:1 (vol:vol) chloroform:methanol. After 24 h, the mixture was filtered and re-extracted twice in a separatory funnel. The filtrate was mixed with 10% NaCl (2.5 to 1, vol/vol) and 2 mg of internal standard (15:0) to quantify individual FA. After 24 h, the layer-containing lipid in chloroform was decanted and dried in a rotary evaporator at 40ºC. Chloroform remaining was evaporated with a N_2 stream. Fatty acids were separated and quantified as FA methyl esters (FAME) prepared by the AOAC (1990) method. The extracted fat was mixed with 1 mL of 1 *M* KOH and 1 mL of 14% (wt/vol) trifluoride boron in methanol. The sample was methylated by incubation at 100ºC for 60 min and after cooling to room temperature, was extracted with 5 mL of hexane. The FAME in the hexane layer were analyzed by GLC (Hewlett Packard 5890 Series II GC, S.A, Barcelona, Spain). All samples were methylated in duplicate, and 0.2 µL were introduced by split injection in a fused silica capillary column (30 m x 0.25 mm ID, BPX 70; 0.25 µm film thickness, Barcelona, Spain). Helium was the carrier gas at 30 cm/sec. Column temperature was initially 150ºC for 1 min, increased 4ºC/min to 200ºC, then held at 200ºC for 10 min. Individual FAME were identified by retention time with reference to FAME standards (Lipid Standard: FA methyl ester mixture#189-19 L-9495; Sigma Chemical Co., St. Louis, MO). The *cis*-9, *trans*-11-CLA, and *trans*-10, *cis*-12-CLA isomers were identified with reference to methyl esters of CLA (O-5507, Sigma-Aldrich, St. Louis, MO). The EPA reference was obtained from Fluka Industriasse (25 CH-9471; Zurich, Switzerland).

2.4. Statistical Analyses

Average daily gain and rumen data were analyzed using mixed-effects ANOVA with repeated measures (SAS Inst. Inc., Cary, NC). The statistical model included block (initial BW), lipid level, lipid source, the interaction between lipid level and lipid source, time, and the interaction between time with lipid level, time with lipid source, and time with lipid source and lipid level, as fixed effects, and bull nested within pen as a random effect to account for any potential dependencies between animals within pen. Time was considered a repeated factor, and the interaction of bull with pen nested within the interaction of lipid level and lipid source (the error term) was subjected to compound symmetry variance-covariance structure. The linear and

quadratic effects of lipid level were evaluated using contrasts. Consumption of concentrate and straw and G:F were analyzed as described above, but pen was the random effect. Carcass quality characteristics, meat fat content, and FA composition were analyzed as described for ADG without the time effect (as there were no repeated measures) and with pen as a random effect.

3. RESULTS AND DISCUSSION

One bull receiving the linseed and low lipid level treatment and another receiving the linseed and high lipid level treatment were removed from the study due to health problems unrelated to the treatments. The fat content of the medium lipid level concentrate was 10% greater than expected (8.9 instead of 8.0%). As no significant time by lipid level or lipid source interactions were observed, only average treatment responses over time are presented.

3.1. Intake, and Animal Performance

Daily concentrate intake (7.24 ± 0.180 kg/d DM) and total DMI (8.40 ± 0.190 kg/d DM) were not affected by lipid level or lipid source (Table 3). To our knowledge, there are no studies that report DMI of young bulls consuming a ration with fat levels near 9% of DM (accounting for the consumption of concentrate and straw), but increasing lipid level (3 to 8% DM) does not consistently decrease intake in growing cattle (Chilliard, 1993). Other studies have not reported any negative effects on feed intake when supplementing beef diets with linseed (Choi et al., 2000; Scollan et al., 2001a; Raes et al., 2004a) or canola seed (Hussein et al., 1995) at lower inclusion rates than in our study. From our study, it can be concluded that this high level of fat, when included as whole seed, has no detrimental effect on DMI.

Straw intake was greater ($P < 0.05$) in bulls fed canola seed (1.25 ± 0.077 kg/d DM) than in those fed linseed (1.10 ± 0.077 kg/d DM), but was not affected by lipid level. Few studies have been conducted in beef cattle where straw and concentrate are both offered for *ad libitum* consumption to evaluate the effects of lipid level and source on straw intake.

Average final BW was 443 kg, and it was achieved after 105 ± 4 d of feeding without differences among treatments. Lipid source did not affect ADG in agreement with other studies conducted with finishing beef fed linseed (Choi et al., 2000; Scollan et al., 2001a; Raes et al., 2004a) or canola seed (Rule et al., 1994; Hussein et al., 1995). However, bulls fed the highest lipid level tended ($P = 0.07$) to grow less (1.27 ± 0.079 kg/d) than the bulls fed the low (1.37 ± 0.079 kg/d) or the medium (1.42 ± 0.079 kg/d) lipid level. The G:F was not affected by treatment.

Table 3. Intake and performance of Holstein bulls fed concentrates containing 2 lipid sources and 3 lipid levels

	Concentrate lipid level, % of DM									
	Low (5%)		Medium (8%)		High (11%)					
	Concentrate lipid source							*P*- value[1]		
Item	Canola seed	Linseed	Canola seed	Linseed	Canola seed	Linseed	SEM	LS	LL	LS x LL
Initial BW, kg	298	299	303	301	305	301	7.4	0.81	0.78	0.92
Final BW, kg	444	436	446	450	439	444	4.2	0.94	0.14	0.28
ADG, kg/d	1.45	1.30	1.34	1.51	1.23	1.32	0.079	0.69	0.07	0.12
Concentrate DMI, kg/d	7.33	7.08	7.46	7.53	6.96	7.08	0.180	0.88	0.11	0.29
Straw DMI, kg/d	1.24	1.03	1.27	1.17	1.24	1.10	0.077	0.04	0.60	0.78
Total DMI, kg/d	8.57	8.11	8.73	8.70	8.20	8.18	0.190	0.36	0.16	0.43
G:F ratio, kg/kg	0.17	0.16	0.15	0.17	0.15	0.16	0.009	0.50	0.49	0.30

[1] LS = lipid source; LL = lipid level.

3.2. Ruminal Fermentation

Average rumen pH was greater ($P < 0.01$) when bulls were fed the high lipid level (6.49 ± 0.088) than when they were fed the medium (6.26 ± 0.088) or low (6.20 ± 0.088) lipid level (Table 4). As expected, total rumen VFA concentration decreased linearly ($P < 0.05$) with lipid level (Table 4), probably as a consequence of the decrease in NFC content as the lipid level in the concentrates increased (Table 1).

Table 4. Rumen fermentation parameters of Holstein bulls fed concentrates containing 2 lipid sources and 3 lipid levels

	Concentrate lipid level, % of DM									
	Low (5%)		Medium (8%)		High (11%)					
	Concentrate lipid source							*P*- value[1]		
Item	Canola seed	Linseed	Canola seed	Linseed	Canola seed	Linseed	SEM	LS	LL	LS x LL
pH	6.28	6.13	6.18	6.35	6.43	6.55	0.088	0.52	0.01	0.19
Total VFA, m*M*	75	75	75	70	60	63	4.7	0.86	0.03	0.72
Individual VFA, mol/100 mol										
Acetate	59.8	59.4	63.4	64.0	67.9	64.9	1.40	0.36	0.01	0.56
Propionate	26.5	26.7	21.0	22.3	19.9	21.1	1.60	0.48	0.01	0.92
n-butyrate	14.9	6.9	14.2	6.9	9.9	8.5	3.00	0.02	0.81	0.45
Acetate:propionate,	1.96	2.01	2.71	2.63	2.89	2.69	0.170	0.45	0.01	0.64

[1] LS = lipid source; LL = lipid level.

[2] Quadratic effect of lipid level ($P < 0.001$).

The inverse relationship between NFC and lipid level, as well as NDF, in the concentrates also could have affected the acetate to propionate ratio in the rumen (Table 4). The acetate to propionate ratio was greater ($P < 0.001$) in bulls fed the high (2.76 ± 0.170) and the medium (2.67 ± 0.170) lipid levels than the low (1.99 ± 0.170) lipid level. Rumen molar proportion of *n*-butyrate was lower ($P < 0.05$) in bulls fed linseed (7.4 ± 3.00 mol/100 mol) than in bulls fed canola seed (13.0 ± 3.00 mol/100 mol).

3.3. Carcass and Meat Quality Characteristics

Carcass and meat quality characteristics are presented in Table 5. Dietary treatments did not affect HCW (233.3 ± 3.30 kg), dressing percentage (52.6% ± 0.48), backfat (71% classified as "3", data not shown), conformation (94% classified as "O", data not shown), ultimate meat pH (5.53 ± 0.042), and LM colourimetric parameters (39.1 ± 0.73, 13.5 ± 0.62, and 4.3 ± 0.47, for L*, a*, and b*, respectively). These results are in agreement with those reported by other authors (Choi et al., 2000; Scollan et al., 2001a; Raes et al., 2004a) comparing the effect of linseed supplementation with other fat sources on carcass characteristics. However, cold carcass weight tended ($P = 0.10$) to decrease in bulls fed the high (224.6 ± 3.21 kg) compared to bulls fed the medium (230.6 ± 3.21 kg) or the low (230.4 ± 3.21 kg) lipid level. Felton and Kerley (2004) observed that HCW tended to decrease as whole raw soybeans inclusion level increased from 0 to 24% in steers fed high-concentrate diets; however, in a second experiment this effect was not observed, and the authors concluded that negative performance effects of whole raw soybeans at inclusion levels of 24% seemed to be minimal.

The area of LM was greater ($P < 0.01$) in the bulls fed the low (29.1 ± 1.29 cm^2) or the medium (31.0 ± 1.29 cm^2) lipid level than in the bulls fed the high (26.9 ± 1.29 cm^2) lipid level. Furthermore, an interaction ($P < 0.05$) between lipid source and lipid level in the LM area was observed, in that LM area was less for bulls fed the low level of linseed than for those fed the low level canola seed, whereas lipid source did not impact LM area when the medium and high levels of lipid were fed. In our study, intramuscular fat content of LM tended ($P = 0.09$) to be lower in bulls fed the medium lipid level (2.34 ± 0.230%) compared with those fed the low (2.94 ± 0.230%) or the high (2.65 ± 0.230%) lipid levels.

Table 5. Carcass and meat quality of LM of Holstein bulls fed concentrates containing 2 lipid sources and 3 lipid levels

	Concentrate lipid level, % of DM									
	Low (5%)		Medium (8%)		High (11%)					
	Concentrate lipid source							*P*- value[1]		
Item	Canola seed	Linseed	Canola seed	Linseed	Canola seed	Linseed	SEM	LS	LL	LS x LL
Carcass										
Hot weight, kg	236.3	232.9	234.1	236.7	231.6	228.4	3.30	0.62	0.23	0.58
Cold weight, kg	232.0	228.8	229.0	231.6	225.2	223.4	3.21	0.69	0.10	0.73
Dressing percentage, %	53.2	53.4	52.4	52.6	52.8	51.5	0.48	0.84	0.34	0.64
LM										
pH	5.52	5.52	5.58	5.53	5.52	5.55	0.042	0.82	0.70	0.69
Area, cm^2	31.2	26.9	30.1	31.8	26.2	27.7	1.29	0.75	0.01	0.04
Instrumental colour[2]										
L*	39.3	38.3	38.3	39.4	39.6	39.3	0.73	0.92	0.68	0.40
a*	13.7	14.4	13.1	12.5	13.7	13.5	0.62	0.95	0.15	0.54
b*	4.8	4.3	3.7	4.2	4.3	4.2	0.47	0.99	0.54	0.62
Intramuscular fat, %	2.92	2.95	2.29	2.38	2.67	2.63	0.230	0.91	0.09	0.96

[1] LS = lipid source, LL = lipid level.

[2]Colour: L = luminosity, a = red colour, and b = yellow colour.

3.4. Fatty Acid Composition of the LM

Fatty acid profile and content of LM are presented in Table 6 and 7, respectively. Several FA (10:0; *cis*-10-15:1; *trans*-10, *cis*-12-18:2; *cis*-8, *cis*-11, *cis*-14-20:3, *cis*-13, *cis*-16-22:2; DHA; 23:0; and *cis*-15-24:1) were not detectable or detected at < 0.01% of total FAME.

The most abundant FA in LM lipid were *cis*-9-18:1 (29.5 ± 1.10%), 16:0 (22.6 ± 0.66%), and 18:0 (19.1 ± 0.77%), as previously reported (Jenkins, 1993). However, major FA in the concentrates (Table 2) were *cis*-9-18:1 (22.1 to 49.5%) followed by LA (from 25.8 to 45.8%) and ALA (from 3.5 to 38.4%). Differences between concentrate and LM in 18:0, LA, and ALA proportions suggest that unsaturated dietary FA had undergone extensive biohydrogenation by rumen microorganisms, although 18:0 is also synthesized *de novo* by elongation of 16:0 (Enser, 1984) or originates from ruminal biohydrogenation of C18 unsaturated FA (Sauvant and Bas, 2001). In our study, it was expected with bulls consuming high-concentrate diets that ruminal pH would be near 5.5, and in consequence rumen biohydrogenation would be reduced (Martin and Jenkins, 2002). However, despite the fact that bulls consumed a high-concentrate diet, the average ruminal pH was > 6.0, and consequently extensive rumen biohydrogenation could be expected after seed coat protection would be lost.

The proportion and content of 8:0 and the content of 12:0 in LM were greater ($P < 0.05$) in bulls fed linseed than in bulls fed canola seed. The content of 13:0 and 17:0, and the percentage of *cis*-10-17:1 in LM decreased ($P < 0.05$) with lipid level. The 16:0 proportion in LM tended ($P = 0.06$) to be greater in the bulls fed the low (22.6 ± 0.66%) or the medium (23.3 ± 0.66%) lipid level than in those fed the high lipid level (21.7 ± 0.66%). In the liver and in the adipose tissue, the major FA synthesized *de novo* is 16:0 (Enser, 1984), although a portion of 16:0 typically is of dietary origin. In our study, the proportion of *de novo* FA (sum of 8:0, 12:0, 13:0, 14:0, *cis*-9-14:1, and 16:0) in LM was lower ($P < 0.05$) in the bulls fed the high lipid level (24.2 ± 0.70%) than in those fed the low (25.5 ± 0.70%) and the medium (26.1 ± 0.70%) lipid levels (Table 6), suggesting less *de novo* FA synthesis in the rumen and in the adipose tissue when high levels of fat were included in the diet. In agreement with our study, an apparent reduction in the synthesis of *de novo* FA ≤ 16:0 has been reported when feeding unsaturated lipids to ruminants (AbuGhazaleh et al., 2002; Martin and Jenkins, 2002; Ueda et al., 2003).

The proportion of 18:0 in LM increased linearly ($P < 0.05$) with lipid level, and tended ($P = 0.06$) to be greater in canola seed (19.5 ± 0.77%) than in linseed treatments (18.3 ± 0.77%). An interaction between lipid level and lipid source in the percentage of 18:0 was observed ($P < 0.01$). The percentage of 18:0 in LM of bulls fed canola seed increased linearly with lipid level, whereas in bulls fed linseed the percentage of 18:0 remained constant. The greater percentage of 18:0 in LM of bulls fed canola seed than in those fed linseed could indicate that rumen biohydrogenation might have been greater in bulls fed canola seed than in bulls fed linseed. The proportion of *trans*-9, *trans*-12-18:2 in LM increased quadratically ($P < 0.001$) with lipid level and was nearly double ($P < 0.001$) in those bulls fed linseed (0.40 ± 0.058%) compared with those fed canola seed (0.22 ± 0.058%). Also, the LM *trans*-9, *trans*-12-18:2 content increased markedly ($P < 0.001$) in the bulls fed linseed (6.7 ± 1.72 mg/100 g of LM) relative to those fed canola seed (4.0 ± 1.72 mg/100 g of LM). The content of *cis*-9, *trans*-11-18:2 in LM tended ($P = 0.06$) to be greater in the bulls fed linseed (6.3 mg/100 g of LM) compared with those fed canola seed (4.9 mg/100 g of LM). The lower content of the biohydrogenation intermediate FA (*cis*-9, *trans*-11-CLA and *trans*-9, *trans*-12-18:2) further reinforces the hypothesis that that biohydrogenation of unsaturated FA to SFA was greater in bulls fed canola seed than in those fed linseed.

Therefore, the results from our study indicate that physical treatments such as crushing, bruising or extrusion are not necessary to ensure rumen microorganisms accessibility to unsaturated FA contents of linseed. It is generally assumed that rumen lipolysis and biohydrogenation in low-roughage diets is lower than in high-roughage diets (Gerson et al., 1985; Sackmann et al., 2003), suggesting that the main rumen biohydrogenating bacteria are cellulolytic. In our study, straw intake was greater in bulls fed canola seed than in those fed linseed, which could have contributed to the apparently greater rumen biohydrogenation in bulls fed canola seed than in bulls fed linseed. In addition, recent studies indicate that diets containing seeds rich in LA and ALA stimulate the production of trans-11-18:1 and *cis*-9, *trans*-11-CLA in the rumen

(Chichlowski et al., 2005). Moreover, several authors (Bauman et al., 1999; Beaulieu et al., 2002; Raes et al., 2004b) reported that the major source of *cis*-9, *trans*-11-CLA is desaturation of endogenous *trans*-11-18:1 in the adipose tissue by the enzyme Δ^9 desaturase. Similar to our study (Table 6), Raes et al. (2004b) feeding Belgian Blue bulls with 17% extruded linseed and 6.80% crushed linseed reported *cis*-9, *trans*-11-CLA values of 0.38 g/100 g of total FA. The content of LA and total n-6 tended (P = 0.06) to be greater in bulls fed linseed (216 ± 27.1 and 229 ± 28.3 mg/100 g of LM, respectively) than in bulls fed canola seed (174 ± 27.1 and 185 ± 28.3 mg/100 g of LM, respectively). However, Scollan et al. (2001a) and Raes et al. (2004a) did not observe an increase of n-6 intramuscular content when supplementing 20% or 2.6% linseed, respectively. The differences in LA and total n-6 content in LM observed in our study could be attributed to rumen biohydrogenation of ALA to LA (van de Vossenberg and Joblin, 2003). Proportions and contents of ALA, EPA, and total n-3 in LM were greater (P < 0.001) in bulls fed linseed than in those fed canola seed, and all increased (P < 0.05) with lipid level. Furthermore, an interaction (P < 0.05) between lipid source and lipid level in the proportion of ALA, EPA, and total n-3 was observed. In bulls fed linseed, the ALA and n-3 proportions and contents in LM increased linearly with lipid level, whereas in bulls fed canola seed they remained constant. These results are in agreement with those reported by other authors (Choi et al., 2000; Scollan et al., 2001a; Raes et al., 2004a). The ratio of PUFA to SFA tended (P = 0.07) to be greater for linseed-supplemented bulls (0.36 ± 0.036) than for those receiving canola seed (0.30 ± 0.036). In all treatments, the ratio of PUFA to SFA was around 0.40, and was greater than values reported for feedlot beef cattle (Enser et al., 1996; Rule et al., 2002). The n-6 to n-3 ratio was lower (P < 0.01) in the bulls fed linseed (10.0 ± 0.01) than in the bulls fed canola seed (26.0 ± 0.01), and decreased (P < 0.05) with dietary lipid level. In bulls fed the high linseed treatment the n-6 to n-3 ratio was close (6.3 ± 0.01) to the 4.0 ratio recommended by Simopoulos (1999) for human health. Furthermore, the amount of ALA in beef increased markedly when linseed was fed, which would help in meeting the daily consumption of ALA recommended by Wijendran and Hayes (2004).

Table 6. FA profile of LM of Holstein bulls fed concentrates containing 2 lipid sources and 3 lipid levels

	Concentrate lipid level, % of DM									
	Low (5%)		Medium			High (11%)				
	Concentrate lipid source							*P*- value[1]		
Fatty acid	Canola seed	Linseed	Canola seed	Linseed	Canola seed	Linseed	SEM	LS	LL	LS x LL
	g/100 g total fatty acids									
8:0	0.06	0.07	0.02	0.07	0.03	0.06	0.018	0.03	0.46	0.41
12:0	0.08	0.08	0.06	0.10	0.07	0.08	0.014	0.19	0.90	0.47
13:0	0.14	0.15	0.11	0.19	0.13	0.14	0.066	0.47	0.97	0.79
14:0	2.22	2.28	2.22	2.25	1.85	2.00	0.19	0.57	0.12	0.93
14:1, *cis*-9	0.31	0.30	0.33	0.34	0.21	0.32	0.042	0.26	0.21	0.36
16:0[2]	22	23	23	23	21	22	0.6	0.57	0.06	0.78
16:1, *cis*-7	0.33	0.58	0.23	0.29	0.25	0.30	0.12	0.23	0.23	0.67
16:1, *cis*-11	2.24	1.99	1.90	2.18	1.66	2.00	0.17	0.35	0.23	0.16
17:0	0.92	0.86	0.90	0.81	0.88	0.81	0.066	0.11	0.70	0.97
17:1, *cis*-10[2]	0.52	0.35	0.68	0.74	0.43	0.45	0.11	0.70	0.02	0.55
18:0	17.8	18.6	19.7	17.9	21.9	18.4	0.77	0.06	0.03	0.01
18:1, *trans*-9	0.58	0.90	0.88	0.75	0.68	1.23	0.40	0.44	0.85	0.66
18:1, *trans*-11	4.51	4.81	4.27	4.50	4.30	4.68	0.48	0.34	0.78	0.98
18:1, *cis*-9	30.1	30.0	29.5	28.3	30.1	28.8	1.10	0.28	0.54	0.81
18:1, *cis*-11	1.78	1.78	1.66	1.67	1.68	1.65	0.065	0.89	0.12	0.93
18:2, *trans*-9,*trans*-12	0.15	0.24	0.22	0.45	0.27	0.50	0.058	0.01	0.01	0.35
18:2, *cis*-9, *cis*-12	10.1	10.2	9.9	11.7	10.0	11.4	0.98	0.17	0.79	0.65
18:2, *cis*-9, *trans*-11	0.25	0.31	0.23	0.26	0.29	0.35	0.042	0.12	0.16	0.95
18:3, *cis*-6, *cis*-9, *cis*-12	0.12	0.08	0.06	0.08	0.08	0.13	0.033	0.63	0.43	0.31
18:3, *cis*-9, *cis*-12, *cis*-15	0.37	0.70	0.46	1.38	0.38	1.90	0.16	0.01	0.01	0.01
20:0	0.11	0.13	0.13	0.11	0.21	0.12	0.011	0.12	0.30	0.35
20:1, *cis*- 11	0.17	0.12	0.17	0.14	0.14	0.10	0.024	0.05	0.37	0.94
20:2, *cis*-11, *cis*-14[2]	0.11	0.08	0.07	0.07	0.10	0.10	0.011	0.37	0.07	0.47
20:3, *cis*-8, *cis*-11, *cis*-14	ND[3]	ND	ND	ND	ND	ND	-	-	-	-
20:3, *cis*-11, *cis*-14, *cis*-17	0.02	ND	0.02	0.01	ND	0.02	0.015	0.70	0.92	0.23
20:4, *cis*-5, *cis*-8, *cis*-11,	2.16	2.40	3.01	2.71	2.74	2.98	0.30	0.80	0.08	0.55
20:5, all *cis*-5, 8, 11, 14, 17	0.09	0.14	0.10	0.26	0.10	0.33	0.049	0.03	0.01	0.07
22:0	0.04	0.04	0.06	0.07	0.08	0.04	0.023	0.66	0.29	0.73
22:1, *cis*-13	ND	ND	ND	ND	ND	ND	-	-	-	-
22:2, *cis*-13, *cis*-16	ND	ND	ND	ND	ND	ND	-	-	-	-
22:5, all *cis*-7, 10, 13, 16, 19	ND	ND	ND	ND	ND	ND	-	-	-	-
24:0	0.07	0.07	0.06	0.09	0.03	0.01	0.030	0.87	0.14	0.72
n-3[4]	0.48	0.84	0.58	1.65	0.48	2.25	0.21	0.01	0.01	0.01
n-6[5]	12.8	12.9	13.2	14.9	13.1	14.9	1.19	0.28	0.51	0.83
PUFA	13.7	14.3	14.2	17.3	14.1	18.0	1.32	0.03	0.23	0.53
Unsaturated	53.9	54.9	53.7	55.8	53.4	57.2	1.29	0.01	0.63	0.41
SFA	43.8	45.1	46.7	44.8	46.6	43.7	1.21	0.36	0.36	0.11

[1] LS = lipid source; LL= lipid level, [2] Quadratic effect of lipid level ($P < 0.01$).

[3] Not detectable or detected at < 0.01/100 g of total fatty acids, [4] Sum of omega-3 (n-3) fatty acids (18:3n-3, 20:3n-3, 20:5n-3, and 22:5n-3); [5] Sum of omega-6 (n-6) fatty acids (18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, and 22:2n-6).

Table 7. FA content of LM of Holstein bulls fed concentrates containing different lipid sources and lipid level

	Concentrate lipid level, % of DM									
	Low (5%)		Medium (8%)		High (11%)					
	Concentrate lipid source							*P*- value[1]		
Fatty acid	Canola seed	Linseed	Canola seed	Linseed	Canola seed	Linseed	SEM	LS	LL	LS x LL
	mg/100 g of LM									
8:0	1.03	1.23	0.54	1.36	0.88	1.28	0.24	0.01	0.72	0.41
12:0	1.39	1.55	1.17	1.65	0.95	1.41	0.24	0.05	0.42	0.75
13:0[3]	2.61	2.94	1.78	1.30	1.72	2.45	0.55	0.61	0.04	0.42
14:0	43.9	47.1	38.6	41.4	33.4	44.1	4.7	0.14	0.32	0.64
14:1, *cis*-9	6.67	6.88	5.87	5.75	3.99	6.06	0.97	0.36	0.20	0.47
16:0	444	478	386	439	380	421	42.7	0.23	0.34	0.97
16:1, *cis*-7	6.5	13.5	4.8	5.6	4.7	6.1	3.2	0.23	0.24	0.56
16:1, *cis*-11	44.8	41.5	36.3	39.9	28.6	38.3	4.7	0.39	0.14	0.41
17:0	18.0	19.3	14.6	15.3	15.1	15.6	1.6	0.54	0.05	0.96
17:1, *cis*-10	9.9	12.4	10.5	12.5	8.4	8.3	2.9	0.53	0.51	0.89
18:0	341	365	319	345	371	349	30.9	0.69	0.63	0.68
18:1, *trans*-9	11.7	9.6	11.3	12.9	11.8	9.1	2.9	0.65	0.83	0.73
18:1, *trans*-11	91	97	76	85	102	91	14.0	0.89	0.43	0.73
18:1, *cis*-9	600	598	496	549	501	549	61.0	0.51	0.37	0.88
18:1, *cis*-11	35.4	36.5	27.7	31.7	29.6	31.1	3.1	0.37	0.09	0.88
18:2, *trans*-9,*trans*-12	3.5	4.7	4.1	9.3	4.7	9.9	1.7	0.01	0.16	0.42
18:2, *cis*-9, *cis*-12	197	214	155	224	169	209	27.1	0.06	0.78	0.62
18:2, *cis*-9, *trans*-11	5.6	6.6	4.0	5.4	5.1	6.9	0.94	0.06	0.20	0.89
18:3, *cis*-6, *cis*-9, *cis*-12	2.24	1.61	1.44	1.64	1.09	2.19	0.58	0.57	0.70	0.21
18:3, *cis*-9, *cis*-12, *cis*-15	7.0	15.0	8.5	25.8	6.7	35.5	4.3	0.01	0.02	0.04
20:0	2.60	2.66	1.95	2.52	2.38	2.59	0.35	0.84	0.10	0.15
20:1, *cis*- 11	3.01	2.37	2.56	2.60	3.07	2.27	0.43	0.19	0.96	0.58
20:2, *cis*-11, *cis*-14[2]	2.16	2.11	1.40	1.46	1.93	1.88	0.29	0.95	0.05	0.97
20:3, *cis*-8, *cis*-11, *cis*-14	ND[3]	ND	ND	ND	ND	ND	-	-	-	-
20:3, *cis*-11, *cis*-14, *cis*-17	0.35	0.22	0.44	0.38	ND[4]	0.39	0.29	0.80	0.76	0.62
20:4, all *cis*-5, 8, 11, 14	42.3	49.7	44.4	52.6	44.1	54.9	8.5	0.16	0.84	0.98
20:5, all *cis*-5, 8, 11, 14, 17	1.54	2.94	1.88	4.92	1.90	6.30	0.85	0.01	0.09	0.22
22:0	1.04	1.38	1.22	1.54	0.98	0.78	0.34	0.57	0.34	0.67
22:1, *cis*-13[2]	0.15	0.27	0.04	0.10	0.50	0.23	0.10	0.72	0.01	0.08
22:2, *cis*-13, *cis*-16	ND	0.06	ND	0.38	ND	0.13	0.14	0.08	0.45	0.47
24:0	1.09	2.02	1.49	2.11	0.78	0.13	0.73	0.53	0.11	0.43
22:5, all *cis*-7, 10, 13, 16, 19	ND	ND	ND	ND	ND	ND	-	-	-	-
n-3[4]	8.9	18.1	10.8	31.2	8.6	42.2	4.4	0.01	0.03	0.03
n-6[5]	243	267	202	281	216	267	28.3	0.06	0.79	0.61
PUFA	262	296	221	326	236	327	39.8	0.02	0.98	0.62
Unsaturated	1071	1114	892	1071	929	1069	102	0.74	0.50	0.78
SFA	851	916	762	847	804	834	78.0	0.35	0.55	0.94
Total FA	1922	2030	1655	1919	1733	1903	217	0.65	0.53	0.32
n-6:n-3, g/g	27.4	14.7	18.6	9.0	25.1	6.3	1.9	0.01	0.03	0.56
PUFA:SFA, g/g	0.31	0.32	0.29	0.38	0.29	0.39	0.036	0.07	0.30	0.51

[1] LS = lipid source; LL= lipid level

[2] Quadratic effect of lipid level ($P < 0.01$)

[3] Not detectable or detected at < 0.01/100 g of LM

[4] Sum of omega-3 (n-3) fatty acids (18:3n-3, 20:3n-3, 20:5n-3, and 22:5n-3)

[5] Sum of omega-6 (n-6) fatty acids (18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, and 22:2n-6).

4. IMPLICATIONS

Feeding high-concentrate diets containing up to 15.5% of whole linseed does not affect negatively dry matter intake. The ruminal pH of young Holstein bulls can be maintained above 6.0 despite consuming high levels of concentrate. In this type of animal, extensive biohydrogenation of fatty acids from the oilseeds takes place despite their theoretical seed coat protection. The content of n-3 fatty acids in meat from young Holstein bulls fed concentrate-based diets can be enhanced by whole linseed supplementation, and the ratio of n-6 to n-3 can be reduced without notable effects on performance and carcass quality.

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CHAPTER VI: EFFECTS OF GLYCERIN SUPPLEMENTATION ON PERFORMANCE AND MEAT QUALITY OF YOUNG HOLSTEIN BULLS FED HIGH-CONCENTRATE DIET

CHAPTER VI:

ABSTRACT

Forty-eight bulls (335 ± 8.6 kg initial BW) were randomly assigned to 4 glycerin levels (0, 4, 8, and 12% of concentrate DM) with the objective of evaluating the effects of glycerin supplementation on performance, ruminal fermentation, metabolism, and carcass and meat quality in Holstein bulls fed high-concentrate diets. Concentrates were formulated to be isonitrogenous and isocaloric (assuming a glycerin ME content of 3.47 Mcal/kg of DM). Concentrate and straw were fed *ad libitum.* Animal BW and feed consumption were recorded monthly. Additionally, rumen and blood samples were collected every month. Bulls were slaughtered after 91 d of study (460 ± 10.9 kg final BW). Hot carcass weight, and carcass backfat, and conformation were recorded. The area, the ultimate meat pH at 24 h, instrumental colour measurements, Warner-Bratzler shear force, and i.m. fat content of LM were determined. Glycerin level did not affect daily concentrate intake (6.89 ± 0.345 kg/d DM), straw intake (1.38 ± 0.069 kg/d DM), total DMI (8.27 ± 0.324 kg/d DM), ADG (1.36 ± 0.087 kg/d), or feed efficiency (0.17 ± 0.009). Similarly, rumen molar proportions of propionic, acetic and butyric acids, and rumen liquid osmolality were unaffected by treatment. However, a lesser rumen pH ($P < 0.05$), and a greater rumen total VFA concentration ($P = 0.09$), serum insulin concentration ($P < 0.05$), and insulin to glucose ratio ($P < 0.05$) were observed in bulls fed 8% glycerin concentrate level compared with those receiving the 0, 4 and 12% levels. No changes were observed in carcass and meat quality. The ME content of glycerin (86% glycerol) can be assumed to be 3.47 Mcal/kg of DM in Holstein bulls fed high-concentrate diets. In addition, feeding concentrate containing up to 12.1% of glycerin does not incur in detrimental effects on performance, ruminal fermentation, metabolism, and carcass and meat quality parameters. In addition, glycerin supplementation does not seem a plausible strategy to prevent a high ultimate meat pH of young Holstein bulls.

Key words: Beef, Glycerol, Rumen, Meat

CHAPTER VI:

1. INTRODUCTION

Glycerin (rich in glycerol) is a by-product from the biodiesel industry (Dasari et al., 2005). The recent increase in biodiesel production has resulted also in an increase of available quantities of glycerin generated from transesterification of vegetable oils (Crandall, 2004). A potential application for glycerin is to serve as gluconeogenic substrate for ruminants (Chung et al., 2007). Glycerol can be converted to glucose in the liver of cattle and provide energy for cellular metabolism (Goff and Horst, 2003). Glycerol enters the gluconeogenic pathway at the level of dihydroxyacetone phosphate and 3-phosphoglyceraldehyde (Leng, 1970; Krehbiel, 2008). Several authors (DeFrain et al., 2004; Chung et al., 2007) have supplemented relatively large doses of glycerol to prevent ketosis in lactating cattle without observing effects on milk production or milk composition.

Glycerin has been used as a gluconeogenic supplement for short-term consumptions. However, glycerin could be included in ruminant rations as an energetic feed ingredient and substitute other feed ingredients such as cereals, and in turn, reduce feeding costs. To our knowledge, there are no studies that evaluate the effects of glycerin supplementation to finishing bulls fed high-concentrate diets on performance, rumen fermentation, metabolism, and carcass and meat quality. The aim of this study was to determine the effects of feeding different concentrate glycerin levels, as an energetic ingredient, on performance, ruminal fermentation, metabolism, and carcass and meat quality from Holstein bulls fed high-concentrate diets.

2. MATERIALS AND METHODS

2.1. Animals, Housing, and Treatments

Forty-eight Holstein bulls were used in a complete randomized design, and managed following the principles and specific guidelines of the IRTA Animal Care Committee. Animals were weighed and distributed in 48 individual pens (1.5 x 3 m). After an adaptation period of 21 d with the animals consuming a control concentrate (0% glycerin), bulls were weighed in two consecutive days and started the study with an average initial BW of 335 ± 8.6 kg and 9.0 ± 0.42 mo of age. Then, bulls were randomly assigned to one of four concentrates containing: no glycerin, a low dose of 4% glycerin, a medium dose of 8% glycerin, and a high dose of 12% glycerin on a DM basis (Table 1). These inclusion levels were chosen after a preliminary experiment conducted to assess pellet quality based on the level of glycerin inclusion (2, 5, 10, 15, and 20%). An attempt was made

to include up to 20% glycerin, but pellet quality was moderately and severely reduced at 15 and 20% inclusion levels, respectively. The glycerin was produced in a soy-diesel facility (Loiret and Haentjens, Barcelona, Spain) and contained 85.7% glycerol, 8.6% water, 5.5% salt, and 0.09% methanol. Glycerin fed in the current study was used as an energetic ingredient; therefore, to obtain four isoenergetic concentrates, the increase in glycerin level was counterbalanced, mainly, by a decrease in cereals content (Table 1). The ME content of glycerin was calculated based on the hypothesis that 1 mol of glycerol would fermented to 0.5 mol of propionic acid, thus providing 0.367 Mcal of ME per mol (Baldwin, 1968). The remaining 50% was assumed to escape rumen fermentation and reach the intestine with an energy content of 4.3 Mcal/kg of DM (NRC, 2001). Therefore, assuming a 95% digestibility and 100% metabolizability, the glycerol escaping rumen degradation would supply about 0.376 Mcal of ME per mol (1 kg of glycerol = 10.87 mols). Based on these assumptions, estimated glycerol ME was 4.03 Mcal of ME/kg of DM, and in consequence the estimated glycerin (86% purity) ME was 3.47 Mcal/kg of DM. All concentrates were also formulated to be isonitrogenous. Furthermore, as glycerin level increased, the inclusion of sodium chloride was reduced, to maintain the same salt concentrations in all four concentrates (Table 1).

Table 1. Ingredient and nutrient composition of the experimental concentrates

	Treatment[1]			
Item	No Glycerin	4% Glycerin	8% Glycerin	12% Glycerin
Ingredients		% of DM		
Corn grain meal	35.9	36.0	36.0	36.0
Barley grain	32.7	24.5	15.5	9.1
Glycerin[1]	-	4.4	8.7	12.1
Corn gluten feed	0.9	1.0	4.5	4.5
Beet pulp	9.1	9.0	9.0	9.0
Wheat straw	1.3	0.9	0.9	1.3
Soybean hulls	7.5	10.8	12.2	13.5
Soybean meal	7.8	8.9	9.1	10.3
Calcium soaps of palm fatty acids	0.4	0.9	0.9	1.0
Palm oil	2.2	1.6	1.4	1.4
Calcium carbonate	0.89	0.89	0.89	1.07
Dicalcium phosphate	0.33	0.36	0.35	0.35
Vitamin premix[2]	0.20	0.20	0.20	0.20
Sepiolite	0.18	0.18	0.18	0.18
Salt	0.60	0.39	0.18	-
Nutrients				
ME[3], Mcal/kg	3.19	3.22	3.23	3.23
CP, % of DM	11.9	11.6	11.9	12.5
Ether extract, % of DM	4.7	4.2	4.8	4.7
NDF, % of DM	20.0	20.4	19.4	19.4
Ash, % of DM	4.3	4.5	4.7	4.8

[1]Contained 85.7% glycerol, 8.6% water, 5.5% salt, and 0.09% methanol.

[2] Every kilogram contained 5,084 IU of vitamin A, and 1,016 IU of vitamin D3, and 50,850 IU of vitamin E.

[3] Calculated according a glycerol EM estimated of 3.47 Mcal per kg of DM

Bulls were fed concentrate and barley straw (3.5% CP, 1.6% EE, 70.9% NDF, and 6.1% ash; DM basis) in two separate troughs (0.6 m x 1.2 x 0.3 m), both *ad libitum*, until 91 d of experiment when they reached a target final BW of approximately 460 kg. Bulls were housed at Cooperativa Agrària de Guissona experimental station (Guissona, Spain).

2.2. Measurements, and Sample Collection

Animal BW, and concentrate and straw consumptions were recorded monthly. Also, rumenocentesis was performed monthly during two consecutive days (24 bulls/d). Rumenocentesis was conducted with a 14-cm 14-gauge needle inserted into the ventral sac of the rumen approximately 15 to 20 cm caudal-ventral to the costocondral junction of the last rib. Rumen liquid pH was measured immediately with a portable pH meter (model 507, Crisson Instruments SA, Barcelona, Spain). Following Jounay (1982), 4 mL of ruminal liquid were mixed with 1 mL of a solution containing 0.2% (wt/wt) mercuric chloride, 2% (wt/wt) orthophosphoric acid, and 0.2% (wt/wt) 4-methylvaleric acid (internal standard) in distilled water, and stored at -20ºC until subsequent VFA analyses. Additionally, 10 mL of rumen liquid were collected and stored at -20ºC for subsequent osmolality analyses as described by Andersen et al. (1994). Blood samples were taken monthly by jugular venipuncture. One blood sample was harvested without additives for insulin concentration analyses, whereas a second blood sample was harvested with sodium fluoride and potassium oxalate for subsequent glucose determination. All blood samples were centrifuged at 1500 x *g* at 4ºC for 15 min, and plasma and serum stored at -20ºC until further analysis.

Bulls were transported to the slaughterhouse after 91 d of study with a final BW of 460 ± 10.9 kg. Truck stocking density was 0.82 ± 0.23 animals/m^2, and transport distance was less than 1 km. At the slaughterhouse, animals were housed in 3 different pens, forming groups of 16 bulls (4 animals from each treatment) with 0.8 animals/m^2, for approximately 3 h before slaughter. Immediately following slaughter, HCW was recorded, and carcass backfat and conformation were graded according to the EU classification system into 1.2.3.4.5 (EU Regulation nº 1208/81) and into (S)EUROP categories (EU Regulation nº 1208/81, 1026/91), respectively. The conformation class designated by the letter “E” (Excellent) describes carcasses with all profiles convex to super-convex, and with exceptional muscle development, whereas the conformation classified as “U” (Very good) describes carcasses with profiles on the whole convex, and with very good muscle development. The carcasses classified as “R” (Good) present profiles on the whole straight, and good muscle development. Carcasses classified as “O” (Fair) present profiles straight to concave, and with average muscle development, whilst carcasses classified as “P” (Poor) present all profiles concave to very concave with poor muscle development. In addition, the degree of backfat describes the amount of fat on the outside of the carcass and in the thoracic cavity. While the class of backfat that classifies as 1 (Low) describes none to low fat cover, the class of backfat classified as 5 (Very High) describes an entire carcass covered with fat and with heavy fat deposits in the

thoracic cavity. Dressing percentage was calculated from HCW. After 24 h of carcass chilling, a sample of LM from the 6th to the 9th ribs was dissected and analyzed immediately for the ultimate meat pH, using a CRISON portable meter equipped with a xerolyt electrode (CRISON pH25, CRISON Instruments SA, Spain). Additionally, instrumental colour measurements for L* (lightness), a* (redness), and b* (yellowness) on the exposed cut surface of the LM muscle between the 6th and 7th ribs after 30 min of bloom time, were measured with a Minolta (CM-2002, Minolta Co. Ldt., Osaka, Japan). The LM area was analyzed by artificial vision (Pomar et al., 2001), and 200-g samples were stored at -20ºC until i.m. fat content and Warner-Bratzler shear force (WBSF) determinations.

2.3. Chemical Analyses

Feed samples were analyzed for DM (24 h at 103ºC), ash (4 h at 550ºC), CP by the Kjeldahl method (AOAC, 1995), NDF according to Van Soest et al. (1991) using sodium sulphite and alpha-amylase, and fat by Soxhlet with a previous acid hydrolysis (AOAC, 1995). Rumen VFA concentration was analyzed with a polyethylene glycol terephtalic acid-treated capillary column (25 m x 0.25 mm ID, 0.25 µm film thickness, BP21, SGE, Europe Ltd., Barcelona, Spain) using GLC (Carlo Erba Instruments chromatograph, CE 5300 HT, Milano, Italy). Rumen liquid osmolality was determined with a vapor pressure osmometer (Vapro Model 5520, Wescor, Inc Logan Utah, USA). Plasma glucose concentration was determined following the hexokinase/G-6-DH enzymatic method (Burrin and Price, 1985), and serum insulin concentration was determined using ELISA (Kit no. 10-1131-01 Mercodia, Uppsala, Sweden).

Intramuscular fat content of LM was predicted using near infrared transmission (NIT) spectroscopy spectrum in the region 850-1050 nm (Infratec 1265 Meat Analyzer, Tecator AB, Uppsala, Sweden), as reported Mach et al. (2008b). The LM sample was homogenized prior to NIT analysis using a food processor (Robot coupe Blixer3, Montceau Les Mines, France). Intramuscular fat content was expressed as g of fat/100 g of muscle. Meat samples obtained for WBSF measurements were wrapped in aluminium foil and cooked to an internal temperature of 71ºC in a convection oven pre-heated to 200ºC. Sample internal temperature was monitored with a data logger and a thermocouple probe inserted horizontally at the steak midpoint. The maximum shear force was determined from 5 replicates (1 cm^2 cross-section, 3-cm long) with fiber direction parallel to the longest dimension of the strip and perpendicular to the direction of the blade (Honikel, 1998), using a texture analyzer (Alliance RT/5 MTS Systems Corp. Eden Prairie, Minneapolis, USA) equipped with a Warner-Bratzler blade.

2.4. Statistical Analyses

Data were normally distributed, with the exception of insulin serum concentration that was transformed to a log-scale to achieve a normal distribution prior any statistical analysis. The values presented herein correspond to non-transformed means; however, SEM and *P*-values correspond to the ANOVA analyses using log-transformed data.

Performance, rumen fermentation, and metabolism data were analyzed using a mixed-effects model with repeated measures (SAS Inst. Inc., Cary, NC). The model included initial BW as a covariate, glycerin level, time, and the interaction between glycerin level and time, as fixed effects, and animal as a random effect. Time was considered a repeated factor, and for each analyzed variable, animal nested within glycerin level (the error term) was subjected to a compound symmetry variance-covariance structure. To analyze rumen fermentation data, the sampling hour within day entered the model as a covariate. Carcass and meat quality characteristics were analyzed as described above but without the time effect (as there were no repeated measures), and final BW was used as a covariate. A Chi-square-test was conducted to evaluate the effects of glycerin level on carcass classification data (categorical variables).

3. RESULTS AND DISCUSSION

Although concentrates were formulated to be isonitrogenous, the CP content of 4% glycerin treatment was 2.5% lesser than expected (11.6 instead of 11.9%), and the CP content of 12% glycerin treatment was 5.0% greater than expected (12.5 instead of 11.9%).

3.1. Intake, and Animal Performance

Average daily concentrate intake (6.89 ± 0.345 kg/d DM), total straw intake (1.38 ± 0.069 kg/d DM), and total DMI (8.27 ± 0.324 kg/d DM) were not affected by glycerin level (Table 2). Ogborn (2006) reported an increase in DMI in pre-partum dairy cattle but a tendency to reduce DMI after calving. To our knowledge, there are no studies that report DMI of bulls consuming a concentrate with glycerin levels near to 12% of DM. Supporting the present results, some studies conducted with lactating cattle fed high-forage diets (DeFrain et al., 2004; Chung et al., 2007) have reported no negative effects on feed intake when supplementing diets with glycerin at similar inclusion rates to the present study.

The concentrate to straw consumption ratio (83:17) was maintained steady among treatments. In contrasts to a previous study (Pyatt et al., 2007) where the replacement of 10% of corn by crude glycerin of a concentrate fed to Angus steers improved ADG and feed conversion, in the current study glycerin level did not affect ADG (1.36 ± 0.087 kg/d) or feed efficiency (0.17 ±

0.009 kg/d). These results support the hypothesis that the assumption that concentrates were isocaloric might be correct. It could also be concluded that glycerin can be used as an energetic ingredient that can effectively substitute cereals in the diets of finishing Holstein bulls, and that the estimated glycerin ME content was 3.47 Mcal/kg of DM. Present results also indicate that some potentially negative glycerin components such as salt (5.5%) and methanol (0.09%) might not exert detrimental effects on DMI and animal growth when glycerin is included in the diets of Holstein bulls up to about 800 g/d.

Table 2. Intake and performance of Holstein bulls fed high-concentrate diets containing different levels of glycerin

Item[2]	Treatment				SEM	*P*-value[1]
	Non Glycerin	4% Glycerin	8% Glycerin	12% Glycerin		
Initial BW, kg	336	335	334	333	8.6	0.99
Final BW, kg	456	468	463	454	10.9	0.67
ADG, kg/d	1.31	1.43	1.40	1.29	0.087	0.71
Concentrate DMI, kg/d	6.80	6.84	7.15	6.78	0.345	0.85
Straw DMI, kg/d	1.38	1.35	1.39	1.41	0.069	0.94
Total DMI, kg/d	8.18	8.19	8.53	8.19	0.324	0.83
G : F ratio	0.16	0.17	0.17	0.16	0.009	0.21

[1] Effect of glycerin level.

[2] Included 48 Holstein bulls

3.2. Ruminal Fermentation

Rumen liquid osmolality was not affected by glycerin level (Table 3). Average rumen pH was lesser ($P < 0.01$) in 8% glycerin bulls than in non glycerin, 4% glycerin, or 12% glycerin bulls. The lesser rumen pH observed with the 8% glycerin treatment was probably due to the numerically greater daily concentrate intake observed with this glycerin level compared to the rest of treatments. Consequently, total rumen VFA concentration tended ($P = 0.09$) to be greater in 8% glycerin bulls than in the animals receiving the other treatments (Table 3).

However, the lesser pH and the greater VFA concentration observed in animals consuming the 8% glycerin concentrate had no impact on animal performance and health. In contrast, no differences were detected in rumen molar proportions of propionate, acetate, and butyrate, nor in the acetate to propionate ratio (Table 3).

In disagreement to these results, previous studies (DeFrain et al., 2004; Trabue et al., 2007) have reported that animals supplemented with glycerol had greater total rumen VFA, greater rumen molar proportions of propionate, and a lower ratio of acetate to propionate than unsupplemented animals. Linke et al. (2004) found that the administration of 1 kg of glycerol as a dietary supplement, an oral drench, and via rumen tube increased rumen propionate.

Table 3. Rumen fermentation parameters of Holstein bulls fed high-concentrate diets containing different levels of glycerin

Item[2]	Treatment				SEM	*P*-value[1]
	Non glycerin	4% Glycerin	8% Glycerin	12% Glycerin		
pH	6.07[a]	6.06[a]	5.68[b]	6.08[a]	0.087	0.01
Osmolality, mmol/kg	398	407	424	399	27.2	0.90
Total VFA, m*M*	139	146	149	132	5.0	0.09
Individual VFA, mol/100 mol						
Acetate	54.6	54.1	50.9	53.3	1.53	0.11
Propionate	35.6	34.3	38.6	35.4	1.46	0.38
Butyrate	9.8	11.6	0.5	11.3	0.63	0.47
Acetate:propionate ratio	1.53	1.57	1.32	1.50	0.132	0.21

[1]Effect of glycerin level.

[2] Included 48 Holstein bulls

[a, b] Within rows, means with different superscripts differ ($P < 0.05$).

3.3. Animal Metabolism

Plasma glucose concentration was not affected by glycerin levels (0.796 ± 0.021 g/L; Table 4). Serum insulin concentration tended ($P = 0.06$) to increase linearly from 0 to 91 d of study (from 1.08 ± 0.116 to 1.22 ± 0.116 µg/L). It has been previously reported that serum insulin concentrations may increase with age (Martin et al., 1979) and carcass fatness (Trenkle and Topel, 1978). Serum insulin concentration was greater ($P < 0.05$) in bulls that received the 8% glycerin treatment (1.37 ± 0.116 µg/L) than in those that received the non glycerin (0.94 ± 0.116 µg/L), or 12% glycerin (0.98 ± 0.116 µg/L) treatments. The insulin to glucose ratio was greater ($P < 0.05$) in 8% glycerine treatment bulls (1.66 ± 0.136 µg/g) than in those fed the non glycerin (1.19 ± 0.136 µg/g), or 12% glycerin (1.22 ± 0.136 µg/g) treatments. In agreement with the results of this study, Ogborn (2006) reported that 500 mL oral bolus of crude glycerin significantly decreased plasma NEFA concentration with no overall significant effects on plasma glucose or insulin in dairy cattle 5 d after calving.

Table 4. Plasma glucose and serum insulin concentration of Holstein bulls fed high-concentrates diets containing different levels of glycerin

Item[2]	Treatment				SEM	*P*- value[1]		
	Non Glycerin	4% Glycerin	8% Glycerin	12% Glycerin		G	T	G x T
Insulin, µg/L[3]	0.94[b]	1.19[ab]	1.37[a]	0.98[b]	0.116	0.05	0.11	0.06
Glucose, g/L	0.798	0.769	0.821	0.797	0.021	0.36	0.40	0.54
Insulin:Glucose, µg/g[3]	1.19[b]	1.56[ab]	1.66[a]	1.22[b]	0.136	0.03	0.001	0.13

[1] Effect of glycerin level, time, and the interaction between glycerin level and time.

[2] Included 48 Holstein bulls

[a, b] Within rows, means with different superscripts differ ($P < 0.05$).

But in contrast to the present results, Parker et al. (2007) reported greater blood glucose concentrations in animals supplemented with 642 g of pure glycerol 48 h before slaughter than in unsupplemented animals, and Goff and Horst (2001) reported an increase in plasma glucose when administering glycerol via an esophageal pump. Probably, the increase in blood glucose concentrations following glycerin supplementation depends on the physiological state of the animal and its energy balance.

3.4. Carcass and Meat Quality Characteristics

Carcass and meat quality characteristics are presented in Table 5. Dietary treatments did not affect HCW (244.2 ± 2.72 kg), dressing percentage (52.6 ± 0.51%), backfat (58% classified as "3"), and conformation (63% classified as "O"). Mach et al. (2008a) reported similar carcass quality data when feeding Holstein bulls high-concentrate diets to a final BW similar to that of the current study. The area (39.8 ± 2.31 cm^2), and intramuscular fat content (3.82 ± 0.451%) of LM were not affected by treatments, although in the present study, it was hypothesized that glycerin supplementation would reduce the acetate to propionate ratio in the rumen, mainly resulting from an increase in rumen molar proportions of propionate, which is a glucose precursor. In addition, glycerol can be converted to glucose in the liver of cattle, thus it was expected that glucose supply would increase in bulls supplemented with glycerol fostering a rise in blood insulin concentrations and lipogenesis. In fact, bulls receiving the 8% glycerin treatment had a numerically greatest blood insulin concentration and also a numerically greatest intramuscular fat content. In addition, glycerin supplementation did not affect colorimetric parameters of LM and ultimate meat pH (Table 5). Furthermore, the average incidence of ultimate meat pH above 6.0 was 33, 58, 75 and 58 ± 3.1% for 0, 4, 8 and 12% glycerin level, respectively.

It is remarkable that high ultimate pH incidences were very high and this could probably be attributed to the pre-slaughter management. In the present study, animals were individually housed during the experiment limiting interactions between each other, and then they were mixed in 3 different pens at slaughterhouse for 3 hours. This could have increased their activity and stress. Supporting present results, Mounier et al. (2006) reported that mixing bulls increases the social behaviour (specially agonistic and sexual interactions), inducing depletion of muscle glycogen content and limiting the extent of muscle acidification. In agreement, Jeleníková et al. (2008) also reported a greater ultimate meat pH in bulls housed in groups than in bulls individually housed (6.29 ± 0.21, and 5.93 ± 0.08, respectively). These results also suggest that concentrate glycerin supplementation at levels up to 12% concentrate does not seem a plausible strategy to prevent a high ultimate meat pH of young Holstein bulls. Although Parker et al. (2007) reported that steers orally dosed at 24 and 48 h before slaughter with glycerol (2 g/kg BW) presented greater glucose concentrations that nonsupplemented steers, and suggested that this elevated blood glucose concentration in the glycerol treated animals may provide a preferential fuel for liver gluconeogenesis, results from present study stated that glycerine supplemented in the concentrate

might not be a feasible strategy to reduce the incidence of ultimate meat pH. Differences between studies might be attributed to the glycerine supplementation strategy (mixed in the concentrate or orally) and to the doses used.

Table 5. Carcass and meat quality of LM from Holstein bulls fed high-concentrate diets containing different levels of glycerin

	Treatment				SEM	*P*- value[1]
	Non Glycerin	4% Glycerin	8% Glycerin	12% Glycerin		
Carcass[2]						
Hot weight, kg	240.6	246.3	245.6	244.4	2.72	0.46
Dressing percentage, %	52.3	53.1	52.9	52.5	0.51	0.66
Backfat classification, %						
1	0.0	0.0	0.0	8.3	1.25	0.65
2	33.3	50.0	33.3	41.7	1.25	0.65
3	66.7	50.0	66.7	50.0	1.25	0.65
Conformation classification, %						
O	58.3	75.0	58.3	58.3	1.50	0.77
P	41.7	25.0	41.7	41.7	1.50	0.77
LM						
pH	5.88	6.17	6.22	6.04	0.14	0.36
L*	33.82	30.42	31.44	33.56	1.22	0.16
a*	13.81	13.16	13.13	13.32	0.69	0.84
b*	1.66	0.23	0.72	1.80	0.58	0.19
Area, cm^2	38.2	40.2	40.5	40.2	2.31	0.86
Intramuscular fat, %	3.65	3.83	4.10	3.73	0.451	0.89
WBSF, kg	4.17	4.01	3.81	3.79	0.364	0.87

[1] Effect of glycerin level.

[2] Included 48 Holstein bulls

Even though the different glycerin levels used in the current study had no effect on WBSF measurements of LM (3.92 ± 0.364 kg), the obtained WBSF results (< 4.0 kg) ensure a tenderness that should result in high consumer acceptance (Miller et al., 2001). The WBSF values observed in the present study, and consequently the good tenderness data, are probably due to the high ultimate meat pH values (Table 6). A likely explanation is that high ultimate meat pH enhances the activity of neutral proteases, e.g. μ-calpains (Kendall et al., 1993). Watanabe et al. (1996) also suggested non-enzymatic causes of the increased meat tenderness in the range 5.8-6.3. However, it is important to take into account that this meat with high ultimate pH presents low palatability (Viljoen et al., 2002; Wulf et al., 2002), and growth of microorganisms to unacceptable levels with development of off-odours, and often slime formation (Gardner et al., 2001), instead of high tenderness.

Table 6. Effect of high ultimate pH on Warner-Bratzler Shear Force of young Holstein bulls fed different crude glycerin levels

Items	Experiments								
	Meat with ultimate pH < 6.0				Meat with ultimate pH ≥ 6.0				
Treatments	0% Glycerin	4% Glycerin	8% Glycerin	12% Glycerin	0% Glycerin	4% Glycerin	8% Glycerin	12% Glycerin	SEM
WBSF, kg	4.5	4.7	5.3	4.3	3.6	3.6	3.4	3.8	0.03

4. IMPLICATIONS

In conclusion, glycerin (86% glycerol) could be assigned an ME estimate of 3.47 Mcal/kg of DM when fed to Holstein bulls receiving high-concentrate diets. In addition, feeding concentrate containing up to 12.1% of glycerin does not incur in detrimental effects on performance, ruminal fermentation, metabolism, animal health, and carcass and meat quality parameters, and thus it could be effectively used as an alternative energy source to substitute cereals in the diet. However, glycerin concentrate supplementation at levels nearly 12.1% does not seem a plausible strategy to prevent a high ultimate meat pH of young Holstein bulls.

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CHAPTER VII: GENERAL DISCUSSION OF RESULTS

CHAPTER VII:

1. ENHANCING CARCASS AND MEAT QUALITY BY MODIFYING THE ANIMAL FEEDING OR MANAGEMENT PRACTICES

1.1. Enhancing carcass and meat quality by modifying the management practices

1.1.1. Enhancing the carcass and meat quality by reducing the incidence of meat with high ultimate pH and extreme carcass bruising

Results from Chapter III, where the effect of animal, transportation, and slaughterhouse practices on beef extreme carcass bruises and ultimate meat pH was studied, stated that the Spanish beef industry is facing an important incidence of meat with high ultimate pH and extreme carcass bruising, causing significant economic losses to the industry. In fact, discounts of up to 150 € per animal could be registered. It is well known that meat with high ultimate pH represents a quality problem in relation to poor shell-life, a dark and dry appearance, and a reduced consumer appeal. Consumers avoid dark coloured meat at the retail point because they associate its dark colour with meat obtained from an old animal or with retail cuts that have been in the display case for too long.

Although 25 factors related to animal, farm, transportation, and animal handling and behaviour at the slaughterhouse, as well as their interactions, have been studied in this thesis, the ultimate meat pH and extreme carcass bruises variability explained by the model was extremely low (R^2= 4.9% and R^2= 13.0%, respectively), which mitigates against the taking of management decisions such as optimizing stocking densities during transportation or during waiting time at slaughterhouse, and applying different handling practices between males and females or between different breeds. Therefore, an important conclusion is that despite significant advances in knowledge in recent years, the mechanisms involved in the *post-mortem* pH decline, are still not well understood. Thus, the incidence of meat with high ultimate pH will probably remain high, and the industry will need to find some feasible strategies to remedy these undesirable traits. Strategies such as modified packaging or marination processes have been proposed (Mach et al., 2007). A less economic solution is to divert the carcasses with high ultimate meat pH to be used in processed products, instead of at the retail level.

However, results from Chapter III might be used to deny that short distance transportation can cause significant fatigue, fear and distress, fasting, dehydration, injuries or detrimental carcass and meat quality, as a consequence of loading, unloading, grading, putting-up to and though the sale-ring, impacts during grading and at specific places when passing to and through the ring, and slips and falls during loading as reported by Gregory et al. (2008), in agreement to Bartos et al.

(1993) and Fernández et al. (1996). In fact, transportation lower than 151 km or 3.46 h (Table 1, Chapter III) was probably too low to exert negative effects on ultimate meat pH or induce extreme carcass bruises. These results also suggested that, in Spanish beef system production, one of the important segments of the meat chain where animal well-being might be further perturbed is the slaughterhouse, where animals are exposed to a wide of challenging stimuli, including: time, handling, novel/unfamiliar environments, food deprivation, water deprivation o with undesirable flavour, noise, changes in structure (e.g. through separation and/or mixing animals from different farms and/or pens), high stocking densities, changes in climatic conditions, and potent blood smell. The importance of slaughterhouse management factors was reflected in that in males and in females the incidence of ultimate high pH increases with waiting time at the slaughterhouse. While the odds ratio of meat ultimate pH slightly increased with waiting time at slaughterhouse (OR= 1.11 and 1.12, for 8.17 to 11.86 and 11.87 to 15.80 h, respectively), after 15.80 h of waiting time at slaughterhouse, the odds ratio of meat ultimate pH doubled (OR= 2.19, Table 8 in Chapter III), in accordance to agonistic behaviour (Chapter III; Table 10). In addition, the stocking density significantly affected ($P < 0.01$) the male ultimate meat between 0.27 to 0.30 animals per m^2, whereas in females linearly decreased, probably as a result of greater agonistic behaviour in males than in females at these stocking densities (Chapter III, Table 11). Although these results suggest important considerations when applying management measures at slaughterhouse, it is noteworthy that the ultimate meat pH and carcass bruises variability explained by the model was really lower, thus making the giving of advice or changes in management not productive.

The complexity of ultimate meat pH phenomenon and its unexplained intra-animal variance is supported by results from Chapter VI, where the concentrate of young Holstein bulls was supplemented with different levels of crude glycerin. Results from Chapter VI indicated that the incidence of ultimate meat pH greater than 6.0 ranged between 33 and 75% after mixing unacquainted young Holstein bulls during 3 h before slaughter, at a density of 0.8 m^2 per animal. Holstein bulls slaughtered at the same age (12 months), with similar carcass weight, conformation and backfat classification, subjected simultaneously to the same slaughterhouse management and practices, presented important differences in the ultimate pH, regardless of the treatments (different levels of crude glycerin). It is widely reported that procedures, which allow mixing of unfamiliar animals increase the risk of meat with high ultimate pH (Warriss, 1990). In fact, Matzke et al. (1985) found that bulls held in individual pens pre-slaughter produced four to five times less meat with high ultimate meat pH than bulls held in pairs. However, housing bulls individually is not a commercial practice in Spain. In order to clarify if mixing at slaughterhouse or if housing individually could be a crucial factor causing the high incidence of meat with high ultimate pH observed in this study, an additional short study (described below) was conducted.

Additional short study: Forty-eight Holstein bulls were distributed in 48 individual pens (1.5 x 3 m), and fed commercial concentrate and barley straw in two separate troughs (0.6 m x 1.2 x 0.3 m), both *ad libitum*, until 95 d of experiment. At day 95 of study, animals were randomly assigned to 1 of the following treatments: 1) To be individually housed at slaughterhouse (n= 16); 2) To be housed in groups of 16 animals at slaughterhouse (n=32). Bulls were transported to the slaughterhouse after 95 d of study with a final BW of 493 ± 5.2 kg. Truck stocking density was 0.8 ± 0.23 animals per m^2, and transport distance was less than 1 km. Animals from different treatments were not mixed in the truck. At the slaughterhouse, animals housed in groups were distributed in 2 different pens, forming groups of 16 bulls with 0.8 animals/m^2, for approximately 3 h before slaughter, while individually housed animals were individually allocated 0.07 animals per m^2. Animal behaviour was registered during the 3 h before slaughter. Whereas animal social behaviours were scored using a 4 continuous behaviour sampling of 15 min each, the general activities of animals were scored using 6 scan sampling of 10 s at 5 min interval (Mounier et al., 2005). Interactions like fighting (when bulls pushed vigorously head against head), social licking (when one bull touched with its tongue any part of another animal's body), attempted mounts (head on the back of another animal), and completed mounts were registered. The general activity of animals were resting, standing, and ruminating. For each pen, the proportion of animals performing a similar activity was calculated.

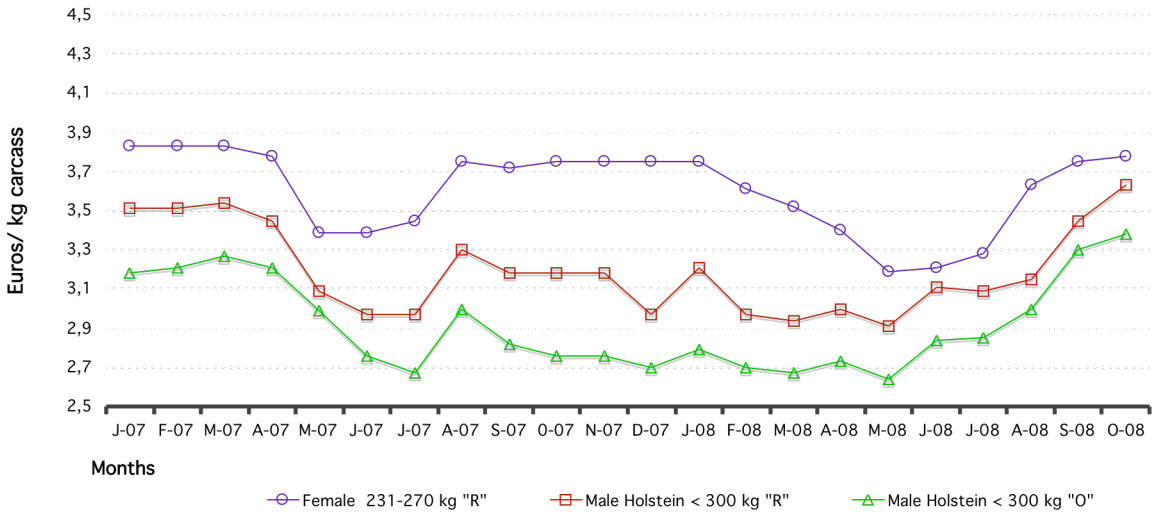
As expected, the mixing at slaughterhouse animals, raised previously individually, significantly influenced the incidence of meat with high ultimate pH. The incidence of ultimate meat pH ≥ 5.8 was greater ($P < 0.001$) in animals mixed at slaughterhouse (21.87%) than in animals individually housed at slaughterhouse and raised previously individually (6.25%). Additionally, the incidence of ultimate meat pH ≥ 6 was greater ($P < 0.001$) in animals mixed at slaughterhouse (16.66), and raised previously individually, than in animals individual housed at slaughterhouse (6.25%), and raised previously individually. But, the results of this experiment reported that mixing unacquainted animals at slaughterhouse might not be sufficient in some animals (78%) to develop high ultimate pH, supporting the complexity *post-mortem* decline of pH and its enormous intra-animal variance. And *vice versa*, it is important to remark that animals housed individually at slaughterhouse could also present high ultimate meat pH (6.25%). Thus, variability exists between animals not only in their perception of the stressor but also in their coordination of response (Moberg, 2001). In addition, the proportion of animal per pen resting, drinking, and ruminating were 0% in both individually and housed in groups. However, in animals housed in group, the observed total number of displacements, social licking, attempted mounts and mounts per pen were 4.04 ± 0.88, 4.09 ± 0.83, 4.20 ± 0.50, and 5.04 ± 0.53 times/5 min per pen, suggesting that animals established a new social hierarchy during the 3 hours before slaughter. It is also likely that effects of social interactions were exacerbated in this short experiment (compared with results previously reported, Chapter III) because of the stressful conditions of lack of previous experience in competitive situations, and the high stocking densities (0.8 animal per m^2). In fact, Dantzer and Mormède (1979) reported that the frequency of fighting has been considered a behavioural

indicator of social stress. It seems, therefore, that even when the energy demand of ruminant muscles increases, as in heavy exercise or in stress, their muscles are not likely to deplete too much glycogen (73% of animals housed in group presented normal meat pH). In agreement, Lambert et al. (1998) demonstrated that fast-walking cattle at speed of 8 km/h over 5 km did not affect glycogen concentration in the LM. In addition, Howard and Lawrie (1956) found that 1.5 h of exercise was insufficient to cause a significant reduction of glycogen levels in the LM of hard driven steers. To further reinforce that the intensity of physical activity and physiological stress responsiveness might not be sufficient in some animals to develop high ultimate pH, results from castrated and intact bulls behaviour are contrasted (Chapter IV). The behaviour of entire bulls and castrates were registered during 4 months before slaughter. As expected, the incidence of animal fighting and displacements per pen was greater in intact bulls than in castrated animals (IRR = 2.86 and IRR= 1.19, respectively). In addition, whereas the frequency of mounts in castrated animals acutely decreased from 60 to 121 days after the castration (from 2.5 to 0.3 times/5 min per pen), in intact bulls it remained constant (3.0 ± 0.56 times/5 min per pen). In the same line, attempted mounts also acutely decreased in castrated animals from 60 to 121 days after the castration (from 2.3 to 0.1 times/5 min per pen), remaining constant in intact bulls (2.9 ± 0.46 times/5 min per pen), indicating that *Burdizzo* castration of pre-pubertal bulls resulted in a reduction of agonistic and sexual behaviours after castration. However, although intact bulls presented greater incidence of agonistic behaviour on-farm during the months previous to slaughter than castrated animals, no differences were reported in the ultimate meat pH (5.5 ± 0.01) and in the incidence of extreme carcass bruises.

1.1.2. Enhancing carcass and meat quality by castration

As reported below, apart from the incidence of high ultimate pH and carcass bruises, currently, the Spanish market is facing other carcass and meat quality problems. In fact, from the study where different variables were studied to clarify factors affecting beef extreme carcass bruises and ultimate pH (Chapter III; Table 2) it was highlighted that 83.3% of Holstein bulls carcasses were classified as “O” conformation, and 30% were classified as “2” in backfat category. Carcasses from Holstein young bulls clearly present less carcass conformation and backfat than that desired in the Spanish market, mainly in Catalonia, causing important industry economic losses. For example, for young Holstein bulls with hot carcass weights lesser than 300 kg, economic losses between carcasses conformation classified as “R” or “O” might be greater than 37€ per animal (Figure 1, Mercabarna 2008). One way to solve these carcass and meat quality problems from Holstein young-bulls might be castration. For decades, the Spanish industry has preferred intact males because of favourable quantitative performances, even if their meat tends to present less quality. However, nowadays, with the globalisation and meat importation from Argentina and Brazil (Mercosur), there is a need to improve the meat quality, leading to higher value meat, and obtaining better market position and prices.

Figure 1. Carcass price during 2007 and 2008 (Euros/kg; Mercabrna, 2008) of females and male



Results from Chapter IV stated that the pre-pubertal *Burdizzo* castration of bulls (8 months of age) is a good strategy; it benefits from faster performance of intact bulls (greater growth rate and feed efficiency) before castration at 8 months of age, and it benefits from the effects of the castration on meat quality characteristics afterwards. That is an important result as a means to improve meat quality of Holstein bulls, which in Spain, are slaughtered at an average age of 12 months. Castration improved the backfat conformation, which determines carcass final price (a premium of 0.05 €/kg of carcass when carcass backfat classification = "3" or superior), intramuscular fat content, which is an important meat quality trait in relation to juiciness, flavour, colorimetric parameters, and also tenderness (the most important quality attribute of meat for consumers, Miller et al. (2001)). Probably, the greater backfat classification and the greater content of intramuscular fat content observed in castrated animals might be associated with the important reduction of anabolic hormones produced by the testes (Adams et al., 1996), which promote muscular development throughout the increase in nitrogen retention (Katz, 2007). It is also important to contrast the intramuscular fat content obtained by castration or feeding strategies, such as oilseeds or glycerin supplementation (Chapter V and VI, respectively). Bulls fed crude glycerin with a concentrate energy of 3.23 ME (kcal/kg) presented greater IMF content than bulls fed diets supplement with oilseeds (2.88 ME, kcal/kg) or a commercial concentrate (2.84 ME content, kcal/kg, Table 1, Chapter VII). Probably, the greater IM fat content in bulls fed concentrate supplemented with crude glycerin compared with others might be the result of the greater concentrate ME and the increased levels of glucose availability and thus, anabolic hormones (insulin), which stimulate lipogenesis. However, to feed animals with concentrate diets containing 3.23 ME (Kcal/kg) is not only expensive, it can also predispose the animals to digestive disorders such as ruminal acidosis, bloat, and displacement of the abomasums (Owens, 1998). However, in the present thesis, animals fed concentrates containing 3.23 ME (Kcal/kg) where individually housed (Chapter VI), avoiding the important effect of feeding concentrate high in energy and competitive social behaviours in the etiology of digestive disorders.

Nevertheless, castration appears to be the better management strategy to increase the beef intramuscular fat content avoiding the risk of digestive disorders. An interesting conclusion from Table 1 (Chapter VII) is that even with concentrates containing 2.84 ME (kcal/kg), is that the intramuscular fat content in castrated animals was two-fold higher than in intact bulls. This concentrate diet containing 2.84 ME (kcal/kg) ensures a reduction of total diet cost and a reduction in the risk of poor appearance or the degree of ruminal acidosis.

Table 1. Meat quality attributes of young Holstein bulls fed different high-concentrate diets

Items	Experiments								
	Chapter VI				Chapter V			Chapter IV	
Treatments	0% Glycerin	4% Glycerin	8% Glycerin	12% Glycerin	5% Lipid level	8% Lipid level	12% Lipid Level	Commercial concentrate (Intact bulls)	Commercial concentrate (Castrated)
Carcass									
HCW, kg	240	246	245	244	234	235	230	283	267
LM									
IMF[1], %	3.65	3.83	4.10	3.73	2.93	2.34	2.65	2.6	4.1
Area, cm^2	38	40	40	40	28	30	27	44	36
Colour[2]									
L*	36.3	32.6	35.9	36.3	38.8	38.85	39.45	34.7	35.3
a*	14.3	13.6	13.3	14.6	14.05	12.8	13.6	14.1	15.5
b*	2.6	1.1	2.4	3.1	4.5	3.9	4.1	6.4	7.2
WBSF[3], kg	4.5	4.7	5.3	4.3	-	-	-	5.6	4.1

[1]Intramuscular fat content

[2]Colour: L*=lightness, a*=redness, and b*= yellowness

[3]Warner-Bratzeler Shear Force

Concerning tenderness, pre-pubertal *Burdizzo* castration appears a good strategy to increase meat tenderness, as tenderness in castrated animals was greater than in intact bulls, at the end of the overall ageing period. Additionally, meat from castrated animals at day 0 of the ageing period showed tenderness values similar to meat from intact bulls after 7 days of ageing, suggesting that beef from castrated carcasses does not need an extended ageing period to achieve an acceptable degree of tenderness (values of WBSF close to 4.0, Miller et al. (2001)). The short storage of carcasses (24-48h) practised in Catalonia is one of the main reasons for the reduced meat tenderness reported in the market. Therefore, if optimal tenderness can be achieved in castrated animals without a long ageing period, it could be a good competitive advantage for the beef industry. It is also interesting to note that, in the present study, *Burdizzo* castration failed in 23% of the cases. This suggests that at 100% of castration efficiency, there would be even a greater difference in muscle tenderness between castrated and intact bulls. Several authors have made an effort to study the effect of the inhibition of anabolic hormones produced by the testes in muscle tenderness. Boccard et al. (1979) and Mc Cormick (1992) reported that castrated animals presented lower concentration of hydroxyproline (a major component of collagen protein) than

intact bulls, as a result of the lack of the anabolic effects of testosterone on collagen synthesis. In addition, Morgan et al. (1993) reported a greater proteolysis in muscle from castrated animals during the first 7 d *post-mortem* than in intact bulls, probably as a result of lower amount of μ-calpain activity remaining at any time *post-mortem*.

The method and age of castration are important factors that affect not only the animal welfare, but also the animal performance and final carcass and meat quality. Castration has been shown to elicit physiological stress, inflammatory reactions (indicated by acute phase proteins), pain-associated behaviour, suppression of immune function, and a reduction in performance (Molony et al., 1995; Fisher et al., 1996; 1997) to varying degrees. Techniques used to castrate male cattle include surgical removal of the testicles (Jennings, 1984), the application of rubber rings or latex bands (Chase et al., 1995) and use of a *Burdizzo* instrument to crush the testicular cords (Robertson et al., 1994). In the present thesis surgical removal of testicles was discarded because of the difficulty of conducting the required post-operative care in commercial farms (cleaning, disinfection, watching for haemorrhages and antibiotic treatment if necessary), and the application of rubber rings was discarded because of greater chronic pain caused by rubber rings up tot 6 months of age compared with *Burdizzo* clamps (Molony et al., 1995; Pang et al., 2006; Thüer et al., 2007). Several studies reported that performance is more negatively affected as the age of castration increases (Table 2, Chapter VII). In addition, Knight et al. (1999b) indicated that the post-pubertal castration of bulls is associated with a reduction in ADG for a short period (45 days) due to castration-associated stress.

Table 2. Effect of the age and method of castration of Holstein bulls on average daily gain and daily dry matter intake reported by different authors

	Castration method[1]	Castration age, months	Days post-castration	Differences in ADG, Kg/d	Differences in DMI, kg/d
Fisher et al. (1996)	BURD	5.5	15-21	⇓	No
Fisher et al. (1996)	SUR	5.5	0-7	⇓	No
Fisher et al. (1997)	SUR	5.0	0-7	⇓	⇓
Earley and Crowe (2002)	SUR	5.0	0-7	⇓	⇓
Ting et al. (2003)	SUR	11.0	0-35	⇓	No
Pang et al. (2006)	BURD	5.5	0-35	No	No
Pang et al. (2006)	BAND	5.5	0-35	No	No

[1]BURD= *Burdizzo* castration; SUR= surgical castration; BAND= castration through rubber rings or latex bands

However, in the present thesis castration was conducted with animals at the age of 8 months because castrations at an earlier age might reduce the overall gain weight and the feed efficiency, and increase the cost of production. Even though there are positive effects of castration on carcass and meat quality, it is important to take into account that castrated animals achieved the slaughter age with a hot carcass weight 16 ± 4.2 kg lesser than intact bulls, representing an economic loss of approximately 50 € per animal. In addition, further research (specially in acute and chronic pain

and stress) will be necessary to ensure that castration is a good method to improve the meat quality in Holstein bulls. Furthermore, the increasingly important ethical concerns of consumers in relation to the intensive hearing methods and management strategies, such as castration, need to take into account. Nowadays EU beef production system is facing farm-level challenges. Indeed, there is increasing social rejection of the currently prevailing intensive production system due to environmental and animal welfare concerns.

1.2. Enhancing meat quality by feeding animals a concentrate rich whole linseed

Effectively, feeding young Holstein bulls with concentrates containing up to 15% of whole linseed in the diet increases the content of n-3 in beef muscle, resulting in a lower n-6 to n-3 ratio, without negatively affects the animal dry matter intake, and presenting no problems with the palatability, nutrient composition, wetness, and metabolic changes associated with high intake of oilseeds. However, bulls supplemented with the concentrate that contained the greatest whole seed content tended (P = 0.07) to grow less (1.27 ± 0.079 kg/d) than the bulls fed the low (1.37 ± 0.079 kg/d) or the medium (1.42 ± 0.079 kg/d) whole seed content, and also the gain to feed ratio of bulls fed concentrate supplemented whole seeds was numerically 27% less than those intact bulls fed a commercial concentrate based in cereal grains (Table 3).

Feeding PUFA rich oilseeds, which were apparently at least partially protected by seed coat from ruminal biohydrogenation resulted in an enhancement of the PUFA in meat with concomitant beneficial improvements in the ratio of polyunsaturated: saturated fatty acids and n-6 to n-3 ratio. Therefore, meat from bulls fed high-concentrate diets containing up to 15% of whole linseed could be considered as a functional food, as result of the beneficial effects of its fatty acid profile on human health and disease prevention. These results are very important to maintain or expand beef market shares in a global market where the competition in the production chain from countries outside the EU and intra-EU is expected to increase in the near future. However, there are still some hurdles in developing and marketing novel functional foods (Arihara, 2006), because such products are unconventional. Along with accumulation of scientific data, there is an urgent need to inform consumers of the exact physiological value of meat and meat products including novel functional meat products. Since food safety is another crucial aspect of food quality, efforts should also be directed to ensure that new functional meat products are safe. In addition, the proliferation of food products and dietary supplements has created an environment of confusion and distrust among health professionals and consumers. Thus, to harmonize the provision of this type of information, many countries worldwide have recently introduced a regulation on the use of nutrition and health claims for foods. In Europe, in December 2006, the regulation on the use of nutrition and health claims for foods was adopted by the Council and Parliament of Europe (European Parliament and Council of Europe, 2006). For the purposes of this regulation, the food claim, nutritional claim, health claim, and reduction of disease risk claim definitions were developed. It must should be noted that medicinal claims for foods, which states or implies that a

product has the property of treating, preventing or curing human disease, are prohibited under national and European Labelling Rules. In order to be permitted to make a medical claim, a product must be called medicine in accordance with the definition in the Directive 2001/83/EC of the European Parliament and of the Council of November 2001 on the Community code relating to medicinal products for human use. The European Food Safety Authority (EFSA) is involve in implementing the new regulation and has published guidance to help companies who want to submit health claims for authorization. The assessment of a health claim by EFSA is the first step in the authorization process. Only those claims, which are scientifically substantiated will be finally authorised for use. The final approval of a health claim is the responsibility of the European Commission and Member States, based on the scientific assessment expressed in the opinion of EFSA Panel. It is the first time that a harmonized approach to authorizing health claims has been established across EU Member Sates. These reinforced controls have increased not only the marginal production costs at the producer, wholesale and retail levels, but also the difficulty and time necessary to certificate meat rich in n-3, as a functional food, in the Spanish market. The opportunities for expansion on the market seem to be quite favourable and the interest of the consumers is quite high, but the diffusion of these products in the community area has been slowed down by some obstacles, including certification and legislation, and the basis of the alimentary traditions and the different cultural heritage that people have acquired.

Thus, although from Chapter V it has been scientifically demonstrated that beef might be enriched with n-3, becoming a functional food, further authorizations needs to be carried out to introduce it into the Spanish market. Furthermore, it is important to consider the purchase cost of whole linseed and its availability in Catalonia. These whole linseeds are produced far from Catalonia, predominantly in UK, India, USA, and Canada (Berglund and Zollinger, 2002), and are subject to high charges, and transportation fees. Furthermore, similar to most grains and oilseeds, the composition of these seeds changes based on variety, and environmental factors (Daun et al., 2003). In addition, the homogeneity of whole oilseed in the concentrate is difficult it, being necessary to consider pre-treatments such as extrusion or crushing. Since there are a considerable number of constraints in the use of these whole seeds, the reduction of beef production costs are uncertain.

Additionally, feeding young Holstein bulls with concentrates containing up to 15% of whole linseed in the diet increases the content of n-3 in beef muscle increases the content of n-3 overall the carcass, whilst consumers are interested only in the prized parts. Is should be considered that it may be more economical for the industry to add controlled amounts of n-3 rich-ingredients to prepared or cooked beef meat, without compromising a finished products taste, texture or aroma. Currently, there are several companies that add controlled doses of DHA from microalgae to perinatal products and infant formulas around the world, ensuring that DHA recovery remains constant throughout the shelf life of the food product. The same companies (e.g. Martek Biosciences Corporation, Columbia, USA) have shown that DHA may also be used in dairy

products such as yogurt, milk, cheese, cheese spreads and ice cream. For example, yogurt may be fortified so as to contain 35 mg DHA per serving. For this application, the DHA oil is added to a small part of milk.

2. REDUCING THE COST OF BEEF PRODUCTION SYSTEM THROUGHOUT FEEDING STRATEGIES

As described above, proper management of feed ingredients and calf purchase is critical to the success of beef production efficiency. Thus an option for increasing intensive beef production efficiency is to reduce animal feeding cost by using alternative feed ingredients. In fact, results reported in Chapter VI suggest that inclusions of crude glycerin up to 10% of total dry matter intake should not incur any negative effects in performance, ruminal fermentation, metabolism, animal health, or carcass and meat quality parameters, and thus it could be effectively used as an alternative energy source to substitute cereals in the diet (Table 3), regardless the content of some potentially negative glycerin components such as salt (5.5%) and methanol (0.09%). The average daily concentrate intake (6.89 ± 0.345 kg/d DM), and total DMI (8.27 ± 0.324 kg/d DM) of animals fed concentrates supplemented with crude glycerin were similar to those reported when young Holstein bulls were fed with high-concentrate diets containing up to 15.5% of whole linseed or 16% of whole canola seed (fat levels nearly 9% of DM, accounting for the consumption of concentrate and straw), and to those reported when young Holstein bulls were fed with commercial concentrate based on cereal grains (Table 3). Indeed, the average daily gain and the feed to gain ratio of bulls fed concentrate supplemented with the crude glycerin was not affected, although the gain to feed ratio of bulls fed concentrate supplemented with crude glycerin, was numerically 27% less than those fed a commercial concentrate based on cereal grains as stated in the Chapter IV. Additionally, although 50% of cereal grains could be replaced by crude glycerin of up to 12% of total dry matter intake, the amount of carcass kg for a given energy intake was less in animals fed concentrate supplemented with crude glycerin (as well as whole seeds rich in fat) than in those fed with a commercial concentrate based in cereals in the Chapter IV.

However, caution must be exercised when comparing data from the different studies. Differences in performance between the experiments might be mainly be due to the different concentrate ingredients, with different values of digestion and/or changes in nutrient availability (ME, CP, NFC), different animal genetic profile, and environmental conditions. Effectively, the ME (kcal/kg) and CP (%) were different between experiments (Table 3). It is important to note that although all bulls studied were Holstein, possible genetic differences could be expected as a consequence of different origin purchase (e.g. Spain vs. Germany).

Table 3. Chemical composition of the concentrates and intake and performance of young Holstein fed different high-concentrate diets

Items	Experiments							
	Chapter III				Chapter IV			Chapter VI
Treatments	0% Glycerin	4% Glycerin	8% Glycerin	12% Glycerin	5% Lipid level	8% Lipid level	12% Lipid Level	Commercial concentrate (Intact bulls)
Crude Protein, %	11.9	11.6	11.9	12.5	14.05	14.15	14.2	14.8
ME, kcal/kg	3.19	3.22	3.23	3.23	2.88	2.88	2.88	2.84
Days of treatment	90	90	90	90	100	100	100	120
Initial BW, kg	336	335	334	333	298	302	303	341
Final BW, kg	456	468	463	454	444	447	440	535
ADG, kg/d	1.3	1.4	1.4	1.3	1.4	1.4	1.3	1.6
G: F, kg/kg	0.16	0.17	0.17	0.16	0.17	0.16	0.15	0.21

The rationality of the use of crude glycerin by-product in ruminant feed in the future will depend on the price in relation to other concentrate ingredients. In the current saturated market conditions, crude glycerin is valued from 35 €/tn to 70 €/tn based on 80% purity (Patzer, 2007). However, in Europe, crude glycerin is more expensive (nearly to 150 €/tn) because of its unsteady supply, and high transport cost. Thus, in view of these prices, using crude glycerin over 15%, as energy supplement for beef cattle in Catalonia may be uneconomical, and does not present an opportunity to reduce the cost of total diet while maintaining animal performance. However, inclusion levels of crude glycerin to nearly 5% could be a good strategy, as the animal performance is not affected, and the concentrate pellet granulation is improved because of its emulsifying and stabilizing properties. Perhaps, as crude glycerin becomes more available, it is likely to become less cost prohibitive as an ingredient in ruminant diet.

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CHAPTER VIII: FINAL CONCLUSIONS

CHAPTER VIII:

Strategies studied in this thesis to improve efficiency of Holstein bulls fed intensively with high concentrate diets allow us to conclude that in our experimental conditions:

Objective 1: To evaluate the effects of different pre-slaughter factors on the incidence of high ultimate meat pH and extreme carcass bruises, in order to elaborate a proposal of management, and technical decisions to reduce meat with high ultimate pH, and to increase the beef production output.

1. The incidence of meat with ultimate pH above 5.8 and 6.0 were 13.89% and 4.02%, respectively, and the incidence of extreme carcass bruises was 2.43%.

2. Males presented 2-times greater incidence of ultimate meat pH ≥ 5.8 and extreme severe carcass bruises than females.

3. Transport distance did not affect the incidence of ultimate meat pH ≥ 5.8 and extreme carcass bruises. However, the stocking density during the transport increased the incidence of extreme carcass bruises above 45% when decreasing stocking density to 1.30 animal per m^2.

4. Waiting time at slaughterhouse above 15.80 h increased the incidence of ultimate meat pH ≥ 5.8 up to 20%.

5. Carcasses conformation classified as “P” presented an incidence of ultimate meat pH ≥ 5.8 up to 30%, and an incidence of extreme carcass bruises close to 18%. In the same line, carcasses backfat classified as “1” presented an incidence of ultimate meat pH ≥ 5.8 up to 42%, and an incidence of extreme carcass bruises close to 11%.

6. Ruminating behaviour at slaughterhouse was 68% greater in females than in males, and slightly decreased as waiting time at the slaughterhouse increased above 12 h.

7. The incidence of mountings at slaughterhouse was 3-times greater in males than in females, and linearly increased as waiting time at slaughterhouse increased above 15.80 hours.

8. **Finally, the ultimate meat pH and extreme carcass bruising variability explained by the pre-slaughter factors studied were very low, 4.1% and 13%, respectively. This makes not feasible to suggest any proposals pertaining to management, technical, or economic decisions, which could lead to improvements on carcass and meat quality observed in intensive beef production systems.**

Objective 2: To improve the carcass backfat classification, as well as meat quality characteristics, during the finishing phase, by pre-pubertal castrating Holstein, without affecting the performance, in order to increase the beef production output.

9. The frequency of mounts and attempted mounts in castrated animals decreased 80% from 60 to 121 days after the castration, whereas in intact bulls it remained constant.

10. The final body weight and the hot carcass weight achieved 121 d after castration were 20 and 16 kg, respectively, less in castrated animals than in intact bulls.

11. A testicular weight and reduction of serum concentration of testosterone was evident after 121 of castration, although 23% of castrated animals did not have a complete testicular weight reduction.

12. Carcass and meat quality were improved by pre-pubertal castration, allowing a differentiated meat quality that could be appreciated in the food market:

    - The carcasses backfat classified as “3” increased by about 27% by pre-pubertal castration. Additionally, the intramuscular fat percentage was two-times greater in castrated bulls than intact bulls.

    - The lightness and redness of meat was enhanced by pre-pubertal castration. Intact males compared with castrates produced darker meat with lower redness and yellowness meat colour values.

    - Tenderness values of meat from castrated animals at day 0 of ageing were similar to tenderness values of meat from intact bulls after 7 days of ageing.

13. **In summary, *Burdizzo* pre-pubertal castration of young Holstein bulls fed high-concentrate diets might be a good strategy to improve carcass and meat quality. However, the castration method efficacy needs to be improved, and carcass weight losses, and castration costs and labour, need to be considered when evaluating the implementation of this management strategy.**

Objective 3: To achieve the differentiation of final quality of carcass and meat in the food market, by supplementation of specific feed ingredients in the ruminant diet, while maintaining or improving animal performance, in order to increase the beef production output.

14. Feeding high-concentrate diets containing up to 15% of whole linseed did not affect negatively animal performance and ruminal fermentation. Also the hot carcass weight, dressing percentage, backfat and conformation classifications, and ultimate meat pH were unaffected by lipid level and lipid source.

15. Proportions and contents of total omega-3 fatty acids in muscle *longissimus* of bulls fed linseed increased linearly with lipid level supplementation.

16. **Feeding high-concentrate diets containing up to 15.5% of whole linseed achieved a maximum of 42 mg of omega-3 fatty acids per 100 g of muscle, and a ratio of omega-6 fatty acids to omega-3 fatty acids of about 6.3 to 1, close to the levels 5 to 1 recommended by the World Health Organisation. This feeding strategy could be a good strategy from the human health point of view to improve the fatty acid profile of meat from Holstein bulls fed high-concentrate diets, however, it is important to consider linseed prices and its availability, as well as, the certification and legislation in order to implement this strategy successfully.**

Objective 4: To reduce the cost of total diet by feeding animals an industrial by-product, while maintaining or improving animal performance, rumen fermentation, metabolism, and carcass and meat quality, in order to reduce the beef production input.

17. Feeding high-concentrate diets containing up to 12.1% of crude glycerin (86% glycerol) did not affect animal performance negatively.

18. The estimate ME of glycerin could be given the value of 3.47 Mcal/kg of DM.

19. Rumen liquid osmolality and rumen molar proportions of propionate, acetate, and butyrate, and the acetate to propionate ratio, were not affected by feeding high-concentrate diets containing different glycerin levels.

20. Carcass and meat quality were not affected by feeding high-concentrate diets containing different glycerin levels.

21. Glycerin might be used as an alternative energy source to substitute cereals in the diet.

22. Glycerin supplementation may not be of use as prophylactic treatment to prevent the incidence of high ultimate meat pH of young Holstein bulls.

23. **In summary, crude glycerin, an industrial by-product, might be included in high-concentrate diets by up to 12.1% as an alternative energy ingredient. However, the reduction of feeding cost through the inclusion of crude glycerin may not be a feasible strategy as a result of the high price of crude glycerin in relation to other concentrate ingredients.**