



UNIVERSIDAD
POLITECNICA
DE VALENCIA

DEPARTAMENTO DE CIENCIA ANIMAL

**El Bienestar Animal y la Calidad de Carne de novillos en Uruguay
con diferentes sistemas de terminación y manejo previo a la faena**

**Animal Welfare and Meat Quality in Uruguayan steers with
different finishing systems and pre slaughter conditions**

**Tesis Doctoral
Ph.D. Thesis**

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A mis padres, Susel y Gustavo

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Resumen

Uruguay es el séptimo país exportador de carne vacuna del mundo y la producción agropecuaria se realiza mayoritariamente en base a pasturas naturales. Los elevados precios internacionales de la carne y la apertura de nuevos mercados en los últimos años, han provocado la intensificación de los sistemas de engorde. Hoy conviven los sistemas pastoriles tradicionales con los de engorde a corral, pasando por una serie de sistemas intermedios con distinto grado de intensificación. El desafío es mejorar la eficiencia del proceso de producción y la calidad del producto, sin afectar las peculiares características de los sistemas extensivos (bajo costo de producción, calidad nutricional de la carne) y sin comprometer el Bienestar animal y el medio ambiente. Es así que el presente trabajo de tesis pretende caracterizar estas diferentes alternativas de intensificación en lo que se refiere a Bienestar animal y calidad de carne, dado el creciente impacto de ambas temáticas en los precios del producto y en las preferencias de los consumidores de los países destino de las exportaciones. Para ello, se realizaron 2 experimentos en Uruguay durante los años 2005-2006 y 2006-2007, respectivamente. En el primer trabajo se utilizaron 84 novillos de raza Británica asignados a cuatro dietas con niveles incrementales de grano, desde una pradera artificial sin uso de suplemento, hasta un tratamiento de alimentación a corral en base a concentrado y heno. La ganancia de peso de los animales aumentó con el nivel energético de la dieta determinando diferentes fechas de faena para todos los tratamientos. Animales de temperamento más calmo tuvieron mayores ganancias de peso independientemente del nivel de alimentación. Los animales confinados mostraron una mayor dificultad para habituarse a las condiciones de producción, según indicadores fisiológicos relativos al estrés. Dichas dificultades pueden atribuirse en parte a la privación de realizar comportamientos probablemente relevantes para el animal, al mayor hacinamiento y/o debido a su menor capacidad de respuesta ante inclemencias climáticas. Dichos animales además mostraron una mayor incidencia de enfermedades derivadas de la dieta, así como una mayor tasa de mortalidad. En general, podría inferirse que el bienestar de estos animales fue menor durante el período de terminación. Los tratamientos intermedios tuvieron mayores pesos de canal caliente y mayor peso de los siete cortes valiosos, pero no se registraron diferencias en el rendimiento de carne entre los diferentes tratamientos. No se obtuvieron diferencias en la proporción de músculo en el corte pistola, pero el porcentaje de grasa de la misma fue mayor en los tratamientos con mayor nivel energético. Los animales provenientes de pastura presentaron grasas con un mayor índice de amarillo, disminuyendo el valor de b^* , conforme aumentaron los niveles de grano en la dieta. Respecto al color de la carne, no hubieron diferencias entre los tratamientos en base a pastura, pero todos presentaron mayores índices de rojo (a^*) que la carne proveniente del

sistema de corral. Los valores de fuerza de corte luego de 7 y 20 días de maduración, fueron menores en la carne de animales provenientes de pastura que en la de los animales de feed lot. A su vez, animales más calmos produjeron carnes más tiernas, en forma independiente al sistema de alimentación.

En el segundo experimento se utilizaron 60 novillos de las razas Braford y Hereford, asignándose equitativamente animales de ambas razas, a las siguientes estrategias de alimentación: a) campo natural con un bajo porcentaje de grano como suplemento y b) un mejoramiento de campo de muy buena calidad. La ganancia de peso no presentó diferencias entre dietas. Considerando las adecuadas ganancias diarias logradas con ambas estrategias de alimentación, que no se registraron enfermedades ni muertes durante el período experimental y que los animales estaban en su medio natural, es posible inferir que el Bienestar animal no se vió comprometido en las alternativas productivas evaluadas. Animales de temperamento más calmo tuvieron mayores ganancias de peso independientemente de la dieta asignada. En este experimento también se evaluaron dos tiempos contrastantes de espera previo a la faena (3 y 15 horas), no habiéndose registrado un efecto del sistema de alimentación sobre la respuesta fisiológica al estrés, en las diferentes etapas estudiadas (transporte por carretera, espera en corrales, traslado al cajón de noqueo). Sin embargo, en forma independiente al sistema de alimentación, animales más calmos presentaron una menor respuesta fisiológica de estrés en las diferentes etapas previas a la faena. El transporte no provocó una respuesta de estrés psicológico o emocional, lo que indica que su efecto negativo sobre el Bienestar animal puede minimizarse con el cumplimiento de buenas prácticas de manejo. Todos los animales presentaron indicios de estrés físico luego de la espera en corrales de matadero y este fue mayor en la espera de 15 horas. Se registró una alta frecuencia de peleas en ambos grupos de faena durante la primera hora de observación del comportamiento en corrales, pero los animales del grupo de 3 horas no tuvieron la oportunidad de habituarse y recuperarse previo al sacrificio. Sin embargo, el hecho de haberles otorgado buenas condiciones de espera, un ambiente calmo y un período de tiempo mayor, permitió que los animales del grupo de 15 horas se recuperaran físicamente, logrando adecuados descensos de pH y consecuentemente mejores valores de color y menores valores de fuerza de corte. Todos los animales sufrieron un importante incremento de los niveles de cortisol en sangre en el momento previo al noqueo, sugiriendo un estado de estrés emocional considerable que deberá estudiarse con mayor profundidad. Un período de 3 horas de espera en corrales previo a la faena no sería suficiente tanto desde el punto de vista del Bienestar animal como de calidad de carne. No se registraron diferencias en las ganancias de peso entre los diferentes sistemas de alimentación, pero los animales suplementados presentaron mayor

peso de canal caliente, del corte pistola, cortes valiosos sin hueso y del Rump&Loin, sin diferencias en el rendimiento de carne entre ambos tratamientos. No se obtuvieron diferencias en la proporción de músculo en el corte pistola, pero el porcentaje de grasa de la misma y el espesor de grasa subcutánea fueron mayores en el tratamiento suplementado. No se registraron diferencias en la fuerza de corte entre las alternativas de alimentación evaluadas, pero tal como ha sido mencionado, la tasa de descenso de pH determinó mejores valores de terneza en los animales que permanecieron la noche en corrales de espera. Los animales de raza Braford presentaron mayores ganancias de peso vivo que los Hereford, mayor porcentaje de músculo en el corte pistola, mayor peso de los cortes valiosos y mayor rendimiento de los mismos. Sin embargo, dicho animales mostraron temperamentos más excitables así como mayores valores de fuerza de corte de la carne.

De ambos experimentos se concluye que el temperamento de los animales constituiría una herramienta muy importante tanto desde el punto de vista productivo como de la calidad de la carne, y en forma independiente del sistema de producción y de la raza utilizada. La inclusión de niveles incrementales de grano en la dieta hasta 1.2 % del peso vivo, permitiría mejorar la “performance” animal sin afectar el Bienestar animal, siempre que se tomen medidas preventivas estrictas respecto a enfermedades provocadas por la dieta. La estrategia de terminación a corral, si bien incrementa los niveles de producción, comprometería el bienestar de los animales y la calidad de la carne producida. Respecto al tiempo de espera en corrales previo a la faena, se considera que se debe otorgar un tiempo prudente a los animales (mayor a 3 y posiblemente menor a 15 horas) que les permita descansar y posiblemente recuperar los niveles de glucógeno del músculo, de forma de lograr una mejor calidad del producto combinado a un adecuado Bienestar animal.

Resum

Uruguai és el seté país exportador de carn de vacú del món i la producció agropecuària es realitza majoritàriament en pastures naturals. En els últims anys els elevats preus internacionals de la carn i l'obertura a nous mercats han provocat la intensificació dels sistemes de producció. Actualment conviuen els sistemes pastorals tradicionals amb els d'engreixament en corral, existint una sèrie de sistemes intermitjos amb distint grau d'intensificació. El present treball de tesi pretén caracteritzar diferents alternatives d'intensificació des del punt de vista del benestar animal i de la qualitat de la carn, degut al creixent impacte d'aquestes temàtiques en els preus del producte i en les preferències dels consumidors dels països destinataris de les exportacions. Per això, es van realitzar 2 experiments a Uruguai durant els anys 2005-2006 i 2006-2007 respectivament. En el primer treball es van utilitzar 84 jònecs de raça britànica, utilitzant quatre dietes amb nivells incrementals de gra ; des d'una praderia artificial sense ús de suplement fins a un tractament d'alimentació en corral utilitzant concentrat i fenc. El guany de pes dels animals va augmentar amb el nivell energètic de la dieta, determinant diferents dies de faenat per a cada tractament. Els animals de temperament més calm van tindre majors guanys de pes independentment del nivell d'alimentació. Els animals confinats van mostrar una major dificultat per a habituar-se a les condicions de producció, segons indicadors fisiològics relatius a l'estrés. Aquestes dificultats poden atribuir-se en part a la privació de realitzar comportaments probablement rellevants per a l'animal, al major confinament i/o a causes de la seua menor capacitat de resposta davant d'inclinemències climàtiques. A més, aquests animals van mostrar una major incidència de malalties derivades de la dieta, així com una major taxa de mortalitat. En general, podria inferir-se que el benestar d'aquests animals va ser menor durant el període de terminació. Els tractaments intermedis van tindre majors pesos de canal calent i major pes dels set talls valuosos. No es van obtindre diferències en la proporció de múscul en el tall pistola, però el percentatge de greix de la mateixa va ser major en els tractaments amb major nivell de gra en la dieta. Els animals de pastures van presentar greixos més grocs, disminuint a mesura que van augmentar els nivells de gra en la dieta. Respecte al color de la carn, no van haver-hi diferències entre els tractaments en pastures, però tots van presentar majors valors d' a* que la carn provinent del sistema en corral. Els valors de força de tall després de 7 i 20 dies de maduració van ser menors en la carn d'animals provinents de pastura que en la dels animals de "feed lot". A més, animals més calms van produir carns més tendres, independentment del sistema d'alimentació.

En el segon experiment es van utilitzar 60 jònecs de raças Braford y Hereford, i les estratègies d'alimentació van consistir en: a) camp natural amb un baix percentatge de gra

com a suplement i b) un millorament de camp de molt bona qualitat. El guany de pes no va presentar diferències entre tractaments sent comparables a les obtingudes en el tractament de pastures de l'experiment 1. Considerant els adequats guanys de pes en aquests dos sistemes, que no es van registrar malalties ni morts durant el període analitzat i que els animals estaven en el seu medi natural, és possible inferir que el benestar dels animals no es va veure afectat per les alternatives productives evaluades. En aquest experiment també es van avaluar dos temps d'espera prèvia al faenat (3 i 15 hores), no havent observat un efecte del sistema d'alimentació en resposta a l'estrés després del transport i de l'espera en corrals a l'escorxador. Animals de temperament més calms van tindre majors guanys de pes independentment del tractament i una menor resposta fisiològica davant de les diferents situacions d'estrés (transport per carretera, espera en corrals, trasllat al caixó de nocheja). El transport no va provocar una resposta d'estrés psicològic o emocional, indicant que es pot minimitzar el seu efecte negatiu sobre el benestar animal amb bones mesures de maneig. Els animals van presentar indicis d'estrés físic després de l'espera i aquest va ser major en l'espera de 15 hores. No obstant, el fet d'haver atorgat bones condicions d'espera i un ambient calm, va permetre que els animals d'aquest grup (espera de 15 hores) es recuperaren físicament, aconseguint adequats descensos de pH i conseqüentment menors valors de força de tall. Els animals que van romandre un període de temps curt en corrals, presentaren valors més alts de pH final i una major duresa de la carn. Això en part podria ser explicat per la major excitabilitat d'aquests animals, havent registrat una freqüència major de comportaments negatius durant l'espera. Tots els animals van sofrir un important increment dels nivells de corticosteroides en sang en el moment previ a la nocheja, suggerint un estat d'estrés emocional considerable que haurà d'estudiar-se amb major deteniment. Tres hores d'espera als corrals abans de començar el faenat no serien suficients des del punt de vista del benestar animal i de la qualitat de la carn. Encara que no van haver diferències en els guanys de pes entre els diferents sistemes d'alimentació, els animals suplementats van presentar major pes de la canal calenta, del tall de pistola, de talls valuosos sense os i "rump&loin". També van tindre major gruix de greix subcutània i major percentatge de greix en el tall de pistola. No es registraren diferències en la força de tall entre les alternatives d'alimentació evaluades però, tal com es va dir abans, la taxa de descens de pH va determinar millors valors de tendresa en els animals que van romandre la nit en corrals d'espera. Els animals de raça Braford van presentar temperaments més excitables que els Hereford, però van aconseguir majors guanys diaris, major percentatge de múscul en el tall de pistola (diferent grau de maduresa), major pes dels talls valuosos, així com valors majors de força de tall en la carn.

D'aquests dos experiments es conclou que el temperament dels animals constitueix una ferramenta molt important tant des del punt de vista productiu com de la qualitat de la carn independent del sistema de producció i de la raça utilitzada. La inclusió de nivells incrementals de gra en la dieta fins al 1.2 % del pes viu permetria millorar la “*performance*” animal sense afectar el benestar animal, sempre que es prenguen mesures preventives estrictes respecte a les malalties provocades per la dieta. L'estrategia de terminació en corral, si bé incrementa els nivells de producció, podria comprometre el benestar dels animals i la qualitat de la carn produïda. Respecte al temps d'espera en corrals previ al sacrifici, es considera que s'ha d'atorgar un temps prudent als animals (major a 3 i possiblement menor a 15 hores), que els poguera permetre descansar i recuperar els nivells de glucogen del múscul, aconseguint una millor qualitat del producte combinat amb un adequat benestar animal.

Abstract

Uruguay is a primary meat exporter being the 7th country in the world in volume, on which its economy is highly dependent. Meat production systems are mainly based on rangeland pastures but during the last years, international meat prices and the opening of new markets have provoked the intensification of fattening systems in order to improve animal performance and carcass and meat quality traits. These intensive systems include a wide range of feeding alternatives between pasture and concentrate utilization. This could involve many differences in terms of carcass and meat quality, which require to be studied. The challenge is to produce a finishing strategy to improve the product, without modifying the peculiar characteristics acquired during extensive grazing conditions (low-cost production and healthy meat for human consumption), and without compromising neither animal welfare nor the environment. Therefore, the objective of the present thesis work was to evaluate the effect of these emerging finishing systems and different pre slaughter procedures on animal welfare, and its effects on meat quality. Two experiments were conducted in Uruguay during 2005-2006 and 2006-2007, respectively. In the first experiment, eighty four Hereford steers were randomly assigned to four diets with increasing levels of grain, from an artificial prairie without supplement to a feed lot system on the basis of concentrate and hay. Live weight gains increased with the level of energy in the diet determining different slaughter dates for all treatments. Animals with higher temperament had higher ADG regardless of diet. According to physiological indicators, confined animals had higher difficulties to get used to the productive conditions. These difficulties could have been due to deprivation of performing relevant behaviours, to crowding and/or due to animal impossibility to face bad weather conditions. Steers from the feed lot had a higher incidence of diet diseases as well as a higher mortality rate. We could infer that the well-being of these animals was lower during the finishing period. Intermediate treatments had higher hot carcass weight and valuable cuts weight, but there were no differences in retail cut yields, between treatments. There were no differences in muscle proportion in the pistola cut between the different finishing strategies, but fat percentage was higher in those treatments with higher level of grain in the diet. Pasture fed animals showed higher fat b values, decreasing with the level of energy in the diet. No differences were found in meat colour between pasture based finishing treatments, but all of them had more red meat than the feed lot. Shear force values were higher in the feed lot system after 7 and 20 days of aging. Calmer animals produced lower shear force values, regardless of finishing strategy.

In the second experiment, 30 Hereford and 30 Braford steers were assigned to two finishing diets: a) native pasture with corn grain as supplement and b) a high quality pasture without

supplementation. ADG did not differ between diets. Calmer animals had higher ADG regardless of diet or breed. Considering ADG, health status and mortality rate, we could preliminary infer that well being was not affected in any diet, during the finishing period. In this experiment, two lairage periods were also evaluated (3 and 15 hours). Calmer animals showed a lower stress response after the different pre slaughter stages (transport, lairage, management and stunning procedure). Transportation did not provoke an emotional stress response, suggesting that its negative effect on animal well being, could be minimised through the implementation of good management practices. All animals showed a physical stress response after lairage, being higher in overnight steers. However, animals from that group had the opportunity to rest during the night and to physically recover. Those animals had a better rate of pH decrease and consequently, lower shear force values. All animals were excited during the first hour in lairage, showing a high frequency of negative behaviours, but animals from the short lairage group did not have the opportunity to rest and to get calm before slaughter. All animals had an important rise in HPA activity in the preslaughter moment, suggesting an emotional stress state that should be deeply studied. A pre slaughter period of 3 hours in the abattoir seemed not to be enough from both perspectives (welfare and meat quality). Supplemented animals had higher hot carcass and valuable cuts weight, but there were no differences in retail cut yield, between diets. There were no differences in pistola muscle proportion between diets, but pistola fat percentage and subcutaneous fat thickness were also higher in the supplemented animals. No differences were registered in shear force values between finishing strategies, but as it was previously mentioned, pH decrease was better in the 15 hours group with the consequent positive effects on meat tenderness. Braford animals had higher ADG during the experiment, higher muscle percentage in the pistola cut, higher valuable cuts weight and a higher meat yield (7CY and RLY). However, they were more excitable and had higher shear force values than Hereford steers.

From both experiments we concluded that temperament could be a very important tool regarding its effects on productivity and meat quality, regardless of finishing strategy or animal breed. The inclusion of increasing levels of grain in the diet until 1.2 % of live weight, could improve animal performance without affecting animal well-being, provided that preventive strict measures are taken in relation to diet problems and diseases. The confined system improved productivity but animals were more stressed, health was compromised and a higher mortality rate was registered. Moreover, meat quality was affected. Regarding to lairage duration, it was considered that animals should have a resting time pre slaughter (higher than 3 hours and probably lower than 15 hours) in order to rest and get calm, probably recovering muscle glycogen contents with the desirable higher meat quality.

CAPITULO I - INTRODUCCIÓN

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1.1. La ganadería en Uruguay

La producción ganadera ha sido tradicionalmente uno de los sectores principales de la agricultura uruguaya. Dentro de ésta, la ganadería vacuna nacional representa el 38% del Valor Bruto de Producción (VBP) pecuario y el 19% del VBP agropecuario (1999), constituyéndose en el 17.5% de las exportaciones totales del país. Es desarrollada por 35.300 productores (82 % del total de establecimientos agropecuarios) que ocupan aproximadamente 12 millones de hás, lo que representa el 70% de la superficie del Uruguay y el 96 % de la superficie dedicada a la producción agropecuaria (15.7 millones de hás) (El Portal Ganadero, 2007). La producción de carne vacuna ha mostrado una tendencia creciente en los últimos 10 años, sobreponiéndose a eventos negativos como la sequía, los brotes de aftosa y la evolución cambiaria desfavorable hasta el año 2002.

1.1.1. Stock bovino

Uruguay es el país con mayor cantidad de vacunos por habitante del mundo: 3.8 cabezas/habitante. El 54% del stock está en poder del 11% de los productores. Los vacunos tienen una participación cada vez más significativa en la producción agropecuaria, debido al crecimiento sostenido de las existencias vacunas (Figura 1) y a la disminución de más del 40% en las existencias ovinas registrado en la última década.

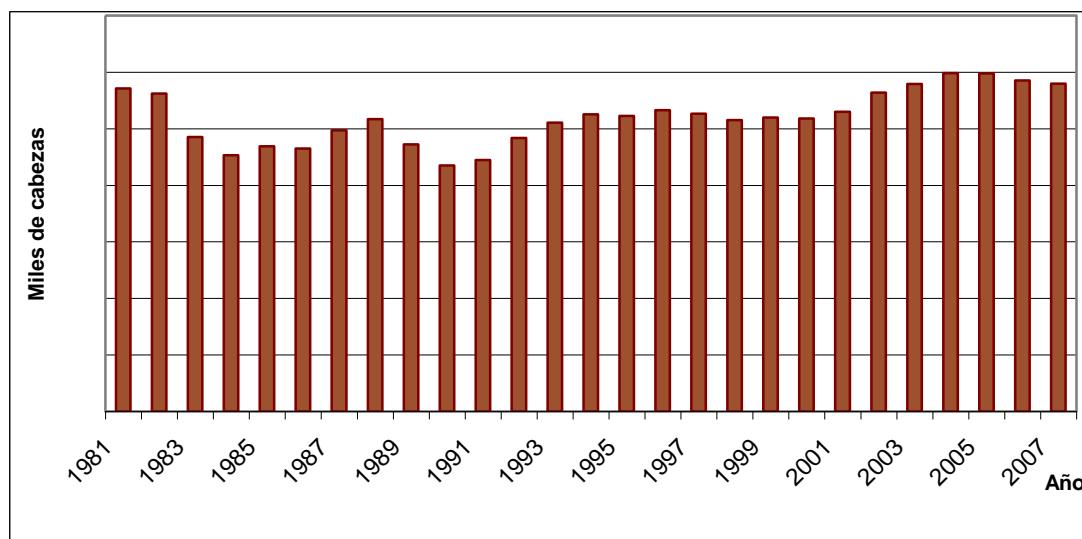


Figura 1. Evolución del stock bovino en Uruguay. Años 1981 al 30 de junio de 2007.
Fuente: INAC, 2008.

1.1.2. Razas

Las razas británicas constituyen el 75 % del rodeo nacional (Shorthorn, Hereford, Aberdeen Angus). Estas son razas precoces, es decir que dependiendo del nivel de alimentación, pueden depositar grasa a cualquier edad alcanzando su punto de terminación. Esto hace que su mercado sea muy flexible, terminándolos para ser vendidos como terneros gordos a los 200-220 Kg. o como novillos a los 400-440 Kg., con todas las opciones intermedias existentes. Su invernada puede ser corta o rápida, dependiendo principalmente del plano nutritivo y por tanto, del nivel de inversiones. Estas razas presentan rendimientos del orden del 56 al 59% en pesos de terminación. Actualmente, la raza Hereford ingresada al país en 1864, es la más relevante. De cada cuatro productores de carne, tres la señalan como su raza principal. Desde entonces ha liderado, habiéndose alcanzado un alto nivel genético y tecnológico (Sociedad de Criadores de Hereford del Uruguay, 2007). Los cruzamientos con razas cebuinas, concretamente la raza Braford está siendo utilizada principalmente en el norte del país, de forma de maximizar la heterosis. Braford es una raza sintética, formada por el cruzamiento de animales de razas cebuinas (Nelore, Brahman o Taba-pua) con reproductores Hereford; interviniendo estas razas en las siguientes proporciones: 3/8 Cebú (37.5%) y 5/8 Hereford (62.5%). La formación de esta raza tiene el propósito de combinar caracteres deseables de cada una de las razas intervenientes y aprovechar las ventajas de las cebuinas en su resistencia al calor y enfermedades (Cole et al., 1963; Crockett et al., 1979).

1.1.3. Orientación productiva

La ubicación geográfica del país y su clima, permiten que el ganado vacuno y ovino permanezca a cielo abierto durante todo el año. Esto, unido a los índices de pluviosidad media y a la existencia de suelos fértiles, permite la producción de una amplia gama de carnes de la más alta calidad en condiciones de pastoreo a cielo abierto, fundamentalmente sobre campo natural. Las diferentes opciones se resumen con la denominación de sistemas pastoriles de producción, los cuales se caracterizan por el pastoreo conjunto de bovinos y ovinos, sin estabulación. Los sistemas productivos, en su diversidad, se pueden agrupar en tres grandes categorías: *cría, ciclo completo e invernada o engorde*, que se distribuyen en el territorio nacional esencialmente en base a la capacidad de uso de los suelos. Del total de productores dedicados a la ganadería, un 63% son criadores, un 22% realizan ciclo completo y un 15% son invernadores especializados (INIA, 2007). La explotación criadora está orientada a la cría de terneros para la obtención de terneros machos castrados o terneras que tienen por destino su comercialización para engorde y terminación por terceros. En la recría, el productor compra los animales al destete y los vende cuando los animales se

hacen adultos (18-24 meses). La explotación invernadora está orientada al engorde de la hacienda destinada directamente a faena, y la de ciclo completo se dedica a la cría, recría y también al engorde de hacienda adquirida o de propia producción.

1.1.3.1. Uso del suelo – Sistemas de Invernada

El engorde de ganado en Uruguay se desarrolla principalmente sobre campos de pastoreo extensivo, ya sean naturales o praderas sembradas de gramíneas y/o leguminosas, según la época del año. Los elevados precios internacionales de la carne y la apertura de nuevos mercados, han provocado en los últimos años, una intensificación de los sistemas de engorde con la consiguiente diversificación en las opciones de producción, de forma de mejorar la eficiencia de producción y atributos de calidad de calidad de la carne. Hoy coexisten en el país, los viejos sistemas pastoriles tradicionales en base a pasturas naturales, con engordes a corral con elevados porcentajes de concentrados. Entre dichos extremos se desarrollan sistemas con distinto grado de intensificación, que en general combinan distintas tecnologías.

Campo Natural

La producción sobre pasturas naturales, ocasiona pérdida de peso en el invierno (15 a 25 kg), grandes ganancias de peso en primavera y moderadas ganancias en verano y otoño. Los animales que se engordan sobre campo natural, tienen generalmente bajas ganancias diarias de peso vivo (0.3 kg/día) durante el año, con pérdidas en invierno, por lo cual necesitan una edad mayor para alcanzar un peso alto y ser faenados. Este tipo de animal está asociado con sistemas de producción extensiva, basados en pasturas naturales, con bajo nivel de insumos e inversión, prácticas simples de manejo, con baja producción de carne (60 a 70 kg/ha/año).

Una pastura natural de calidad media permite ganancias de peso de 0.25 kg/día y una producción de carne de alrededor de 80 a 100 kg/año. Los campos de buena calidad permiten mayores ganancias de peso (0.35 kg/día) y una producción de hasta 150 kg/año.

Pasturas mejoradas

La producción sobre pasturas mejoradas presenta varias opciones tecnológicas. Está basada en la obtención de ganancias en el invierno lo que permite a las existencias tomar mejor ventaja en primavera y verano para acelerar el crecimiento. Un novillo a menudo alcanza 380 kg a la edad de dos años y con menos de un año de engorde puede ser faenado. Un campo mejorado mediante siembra en cobertura muestra un incremento sustancial en ganancia diaria (0.6 a 1.0 kg/día) y en producción de carne por hectárea

(mayor a 250 kg/año). Las pasturas cultivadas, anuales y perennes, permiten ganancias promedio de 0.6 a 1.3 kg/día y producciones de carne desde 240 a 400 kg/año. El manejo correcto del sistema de pastoreo es la clave para aprovechar la alta productividad y calidad del forraje producido y para obtener alta producción de carne por hectárea.

Sistemas intensivos

Se utiliza la suplementación con ración y/o grano y existe un bajo número de productores que realizan invernada a corral, dada la existencia de un mercado que demanda este tipo de producto. Las tecnologías de alimentación y suplementación estratégica sobre los campos (con concentrados, reservas forrajeras o pastoreo racionado de pasturas cultivadas), permiten superar las limitaciones de energía y proteína del forraje. Sus efectos se reflejan en mejoramientos de la ganancia diaria pero fundamentalmente en una mayor producción de carne (450-800 kg/ha). Las principales fuentes de concentrados de energía usados son granos y sub-productos agro-industriales producidos en el país como maíz, sorgo, trigo, cebada, avena, afrechillo de arroz, alimento de gluten, pulpa de citrus. Los concentrados de proteína más usados provienen de la producción agro-industrial: harina de pescado, expeller de girasol, afrechillos, harina de soja. En sistemas intensivos, se alcanzan pesos mayores a 350 kg, cuando los novillos tienen un año de edad, e inclusive pesos de faena superiores a 440 kg. En el caso de engorde a corral, los animales se alimentan con grano de excelente calidad hasta llegar a la calidad de carcasa deseada para ser enviados a faena. El novillo que deja el feed-lot lo hace por lo general con un peso entre 450 kg y 620 kg con una edad que varía entre 14 y 30 meses. El tiempo que abarca este tipo de alimentación es como mínimo de 90 días, al final del cual se entrega un lote de animales de muy similares condiciones. El aumento diario de peso del novillo en feed lot con un manejo alimenticio eficiente, puede llegar a 1.7 kg (INIA, 2007).

1.1.4. Industria

La industria cárnica del Uruguay es un componente fundamental de la economía del país. En la actualidad se destina casi un 80% de la producción nacional a la exportación, por lo que el crecimiento del sector demanda tecnología para el aumento de su eficiencia y la mejora en las características del producto, de manera de atender las exigencias del mercado internacional y mantener el compromiso con los países compradores. El ganado es faenado en las plantas procesadoras aprobadas, las cuales cuentan con un nivel tecnológico de avanzada, mano de obra altamente calificada y reúnen los más rigurosos estándares

internacionales de higiene y de control de procesos (HACCP)¹. El Uruguay cuenta con 40 plantas frigoríficas habilitadas para exportación de las cuales 17 tienen habilitación para la Unión Europea y 15 a Estados Unidos (INIA, 2007). Con la finalidad de asegurar consistencia en la calidad y el cumplimiento de las especificaciones del producto requeridos por los compradores, el Instituto Nacional de Carnes desarrolla un Programa de Control de Calidad Comercial que comprende todas las carnes exportadas. Asimismo, los procedimientos sanitarios, los sistemas de proceso y su observación, son sometidos a revisiones regulares o periódicas por veterinarios de los países importadores.

1.1.5. Faena y exportaciones

En el año 2006, la faena de bovinos fue de 2.588 miles de cabezas, equivalente a 1151 millones de toneladas en pie (Figura 2). La faena ha crecido a un ritmo que supera el 2.6% promedio anual durante los últimos 15 años. La composición de la misma es en promedio: 51 % de novillos, 46 % vacas y el restante 3 %, terneros y toros. La cantidad de animales faenados provenientes de sistemas intensivos de engorde a corral no supera el 8% del total.

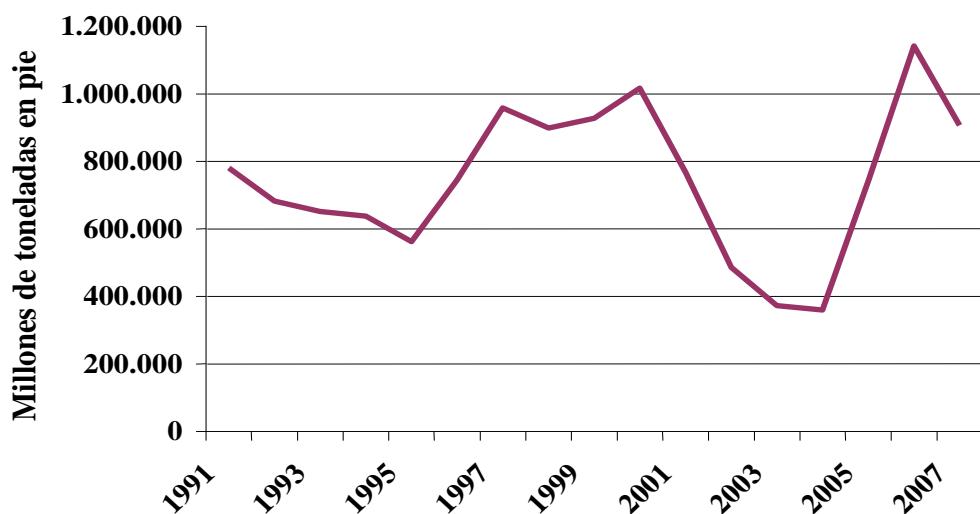


Figura 2. Evolución de la faena bovina en Uruguay. Años 1991 - 2007.
Fuente: INAC, 2008.

Uruguay ha tenido un crecimiento en las exportaciones acompañando el incremento en la producción y presenta un perfil amplio en los mercados internacionales en lo que se refiere a carnes rojas. Entre 1996 y 2004, las ventas al exterior se incrementaron en más del 100%.

¹ El HACCP es una herramienta de gestión de la calidad en la producción de alimentos (especialmente en la etapa de transformación industrial) que apunta al análisis de riesgos y control de puntos críticos.

En el año 2006, las ventas ascendieron a 881 millones de euros, constituyendo la carne vacuna el 86 % y la carne ovina el 4.3 % (INAC, 2008). En el año 2006, se exportaron 477.740 toneladas (peso carcasa) de carne vacuna (92 % refrigerada, sin hueso) y 17.000 toneladas de carne ovina a más de 60 países del mundo (INAC, 2008). Estados Unidos y los países de Europa (UE y otros países) fueron el principal destino con compras equivalentes al 40% del total, respectivamente, según informes de INAC. Esto se tradujo en la cifra registrada más alta de exportaciones, tanto en los volúmenes operados como en los montos recaudados. En su conjunto, en los últimos años, los países del NAFTA (Estados Unidos, Canadá y México) recibieron la mayoría del total de exportaciones cárnicas uruguayas. Cifras preliminares del ejercicio 2007 (INAC, 2008) indican que durante ese año, el 56 % de las exportaciones fue a los países del NAFTA, manteniéndose un importante volumen a la UE y otros países (36 %) (Figura 3). De ese 36 %, el 34 % fue a la UE y el 66 % a otros países). Entre esos destinos se destaca a Rusia, Israel, Sud Africa y Argelia (Mathews y Vandever, 2007). Dentro de la Unión Europea, Reino Unido es el cliente mayoritario de las carnes uruguayas, alcanzando el 50 % de las exportaciones con 23.348 toneladas de peso carcasa en el año 2007 (Figura 3). El acceso a nuevos mercados ha dinamizado las corrientes exportadoras y la comercialización de haciendas, a través de demandas sostenidas por una amplia diversidad de productos.

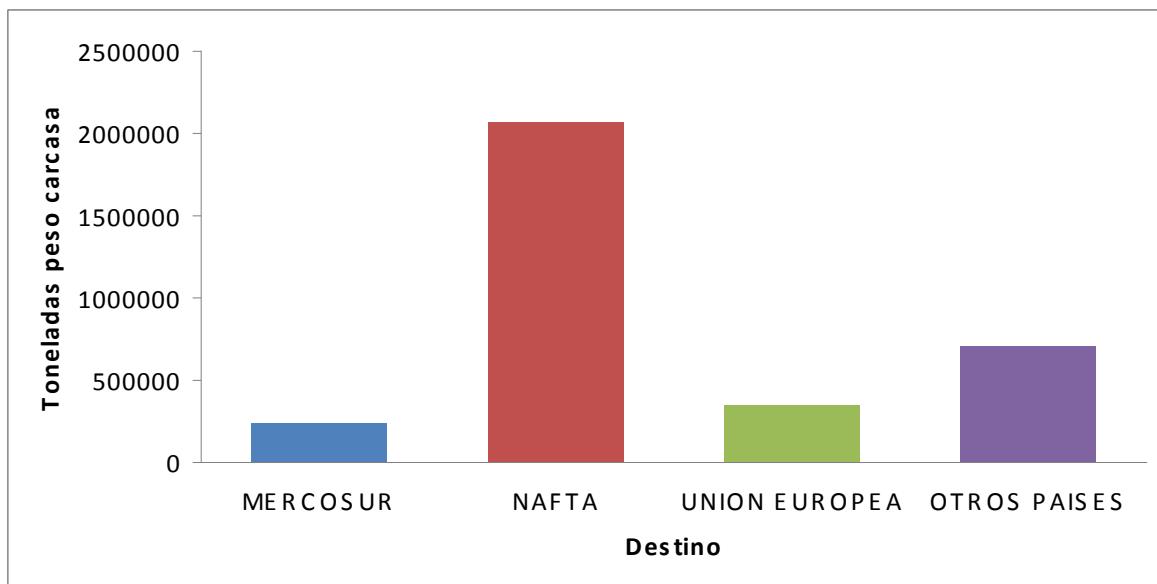


Figura 3. Volumen de exportaciones de carne Uruguaya según destino. Año 2007.
Fuente: INAC, 2008. Cifras preliminares

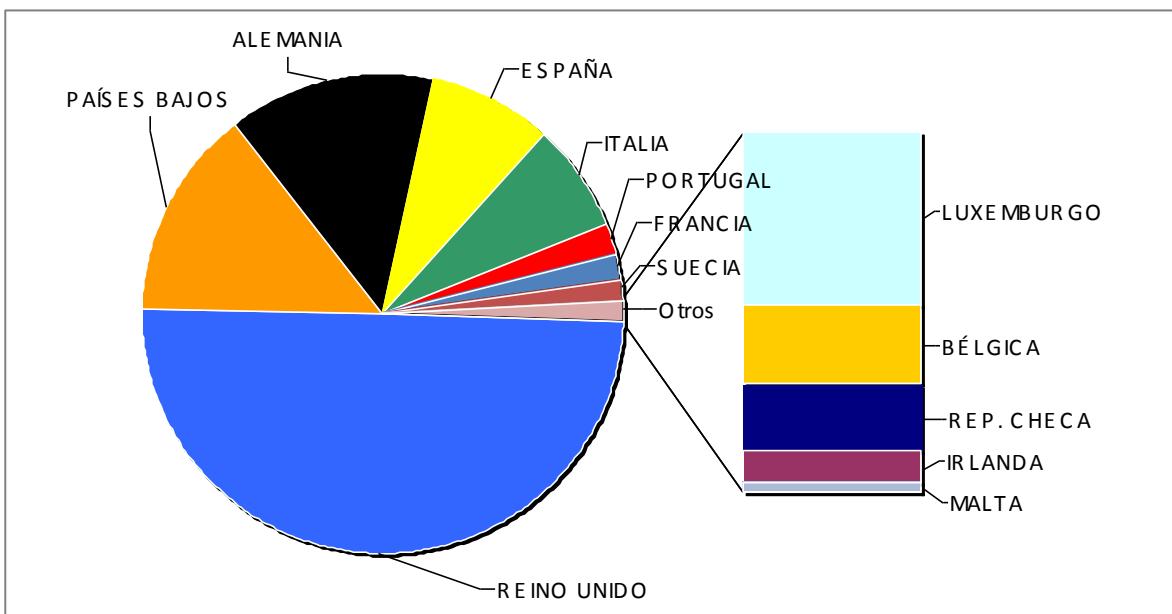


Figura 4. Exportaciones de carne Uruguaya por destino dentro de la Unión Europea. Año 2007.

Fuente: INAC, 2008. Cifras preliminares.

1.1.6. Status sanitario

Uruguay exhibe un status sanitario de alta confiabilidad en los mercados internacionales, está libre de todas las enfermedades de la lista A de la Organización Mundial de la Sanidad Animal (OIE) y se encuentra en plena campaña de erradicación de Tuberculosis y Brucelosis bovina. El actual status sanitario del país es de “país libre de Fiebre Aftosa con vacunación”. En lo referente a la Encefalopatía Espongiforme Bovina (BSE), en la 73^{ra} Asamblea General de la OIE, Uruguay ha sido ratificado como “País Provisionalmente Libre de Encefalopatía Espongiforme Bovina” que es la máxima categoría que tiene la OIE, integrada solamente por cuatro países en el mundo (Argentina, Singapur, Islandia y Uruguay). En el año 2005, Uruguay se presentó ante la OIE, solicitando ser declarado como “País Libre de Encefalopatía Espongiforme Bovina”. En ese sentido, se ha ampliado la reglamentación para darle mayor seguridad y se prohíbe la utilización de alimentos integrados de harinas de carne y hueso de mamíferos.

1.1.7. Trazabilidad individual del ganado vacuno

El Sistema Individual de Registro Animal (SIRA) en Uruguay, fue creado a través de la Ley 17.997. A partir del 1º de septiembre de 2006 comienza en el país con carácter obligatorio, la identificación y registro individual de todos los terneros nacidos en territorio nacional, así como el registro individual de los movimientos con o sin cambio de propiedad. Uruguay constituye un caso casi único en el mundo, al adoptar el sistema de trazabilidad en la

cadena cárnica cubriendo el 100% de su stock bovino. La Ley Bioterrorista, la legislación europea y la eficiencia en los procesos productivos, hacen que sea indispensable conocer la trazabilidad de toda la producción para seguir siendo competitivo. En la producción moderna no se concibe un producto sin sus datos de trazabilidad (SIRA, 2007; Diario Oficial de las Comunidades Europeas, 2007). Luego de los impactos que produjo la BSE en 2003, los consumidores demandan mayor información asociada a los productos cárnicos bovinos. En ese sentido, y como forma de asegurarse que los alimentos a ingerir no sean nocivos para la salud humana, a partir de 2010 la Comunidad Europea no importará carne bovina de países que no tengan afianzados sus sistemas de trazabilidad.

1.1.8. Auditoría Nacional de Carne Vacuna

Años 2002 - 2003, 2007- 2008

En el año 2002 se realizó la 1^a Auditoría de Calidad de la Cadena Cárnia Uruguaya, por parte de INIA, INAC y la Universidad de Colorado State, con la participación, en el taller de discusión final, de representantes de todos los eslabones de la misma. Allí se estimaron los montos que la cadena de la carne deja de percibir debido a diferentes factores, tanto en el sector bovino (Tabla 1) como en el ovino.

Tabla 1. Pérdidas de valor por causa de los defectos identificados en la Auditoría de Carne Vacuna (euros por animal).

Defecto	Pérdida por animal (€)
Cortes oscuros	10.49
Edad (madurez excesiva)	6.26
Defectos del cuero	3.15
Decomisos	1.69
Hematomas/Machucamiento	0.74
Lesiones en sitios de inyección	0.51
Faena de vacas preñadas	0.41
Grasa amarilla	0.30
Total	€ 23.55

Fuente: INIA, 2003.

En base a dichos cálculos, ante una faena anual de 2.588.000 de cabezas bovinas, (año 2006, Fuente: INAC), la Cadena Cárnia Bovina del Uruguay, en su conjunto, dejó de percibir en ese año una cifra cercana a los 61 millones de euros.

Se destaca la relevancia de las pérdidas debidas a factores directamente atribuibles o relacionados al manejo y al bienestar de los animales en lo que tiene que ver con la calidad de las canales, así como en el proceso de transformación de músculo a carne (defectos en

el cuero, hematomas, lesiones en sitios de inyección, decomisos, cortes oscuros). La preocupación que surgiera en base a dichos resultados, ha sido el punto de partida de diversas recomendaciones y medidas de control a nivel nacional, en las diferentes etapas o eslabones de la cadena de producción de carne. Dicho esfuerzo ha sido reflejado en los resultados de la segunda Auditoría Nacional de Calidad de Carne realizada en Uruguay durante los años 2007 y 2008 por INIA e INAC, recientemente finalizada en el mes de noviembre de 2008. En este sentido se destaca (INIA, 2008):

- Incremento del porcentaje de canales sin hematomas (39.6 % en 2002-2003 vs 68.2 % en 2007-2008).
- Importante disminución en la incidencia de hematomas de tipo mayor (35.4 % en 2002-2003 vs 14 % en 2007-2008) y de tipo menor (25 % en 2002-2003 vs 17.8 % en 2007-2008)
- Importante disminución en decomisos de lengua, cabeza y vísceras y pequeña disminución en decomiso total de hígado
- Disminución en el número de canales con pH mayor a 5.8 (22.7 % en 2002-2003 vs 14.7 % en 2007-2008)
- Disminución de la presencia de cortes oscuros (18.8 % en 2002-2003 vs 11.1 % en 2007-2008).

1.1.9. Situación del Bienestar animal en el mundo

La sensibilización acerca de los temas relacionados al BA se ha consolidado y aumentado en los países desarrollados, constituyéndose en un importante elemento de presión para el sector ganadero. Las políticas referidas al Bienestar animal son un aspecto de gran preocupación en la opinión pública y este aspecto ya no es solamente inherente a los países desarrollados. Dado el incremento de esa conciencia social, se han realizado algunas correcciones o mejoras a las legislaciones existentes. En la Unión Europea, a partir del año 1997 en un anexo al Protocolo de Amsterdam (1997), se le brinda a los animales el status de “animales que sienten”, dejando atrás el concepto de mercancías o productos. Allí se establece como legalmente obligatoria la consideración del Bienestar animal al momento de dictar políticas en las áreas de agricultura, investigación, transporte y mercado interno. El objetivo general es evitar a los animales todo dolor o sufrimiento innecesario y proporcionar un manejo que les permita desarrollar sus necesidades biológicas específicas.

Actualmente existe una comisión específica de la UE, responsable de analizar y asesorar sobre este tema. La misma se encuentra en la etapa de difusión de un “Plan de Acción” que

fue implementado a partir de 2006 y funcionará hasta el año 2010, en el que se ajustarán medidas relativas al BA, y el cumplimiento de normativas incrementales será obligatorio.

Dicho Plan se ha planteado 5 objetivos principales:

- 1- Incremento de los requerimientos mínimos de Bienestar animal
- 2- Capacitación de cuidadores y sensibilización del público en general
- 3- Implementación y cumplimiento riguroso del principio de las “3R” a nivel experimental: (replacement, reduction, refinement).
- 4- Estandarización de los indicadores de BA
- 5- Continuar con estrategias y acciones internacionales para el logro de la sensibilización mundial sobre el BA.

En este contexto, se encuentra en marcha un Proyecto de Investigación Europeo, llamado “*Welfare Quality*”, en el cual participan 39 instituciones y universidades representando a 13 países de Europa, habiendo incluido en una segunda etapa, a países como Chile y Uruguay. Dicho proyecto intentará estandarizar todas aquellas variables que comprometerían el BA en los diferentes niveles de producción y así hacer posible su cuantificación y la realización de controles y auditorías.

Cada vez es más requerida la transparencia y el liderazgo a nivel internacional. La importancia de esta cuestión y la implicación de la Comunidad Científica en sensibilizar al resto del mundo sobre ello, han sido destacadas en la Primera Conferencia Mundial sobre Bienestar animal organizada por la Organización Mundial de Sanidad Animal (OIE) en febrero del año 2004, con la participación de 150 países. De la misma surgieron lineamientos que dieron lugar a estándares, aprobados en mayo de 2005.

En respuesta a dicha reunión de la OIE y de forma de establecer recomendaciones de BA a nivel nacional, surge en Uruguay la constitución de un Grupo Técnico específico que funciona en la órbita del Ministerio de Ganadería, Agricultura y Pesca (MGAP) y el cual es integrado por INIA. Actualmente existen normas y estándares que tienden a asegurar el BA en diversos países: Unión Europea, Estados Unidos, Nueva Zelanda, Australia, Canadá, Argentina, Chile y Uruguay. Las mismas parten de la base que el bienestar de los animales se cumpliría en la medida que se respeten las 5 libertades planteadas por el “Farm Animal Welfare Council” (FAWC, 1992):

- 1) libre de hambre, sed y malnutrición
- 2) libre de dolor, lesiones (heridas) o enfermedades
- 3) libre de miedo y angustia

- 4) libre de tener incomodidad (molestias físicas)
- 5) libre de manifestar su comportamiento natural

Dichas categorías no son definitivas ni independientes una de otra. Sin embargo, pueden constituir un marco teórico de referencia para la determinación, monitoreo y control del BA.

1.1.10. El caso de Uruguay

Para países como Uruguay, cuyo desarrollo económico depende en gran medida del crecimiento de las exportaciones, las exigencias de los consumidores de los países de mayor poder adquisitivo, marcan la dirección de la producción y determinan las características de los productos. Esto determina la necesidad de conocer tanto la calidad de los productos generados en los sistemas tradicionales y emergentes de producción, como las características de los procesos en que esos productos se generan, desde el punto de vista del Bienestar animal y de la sostenibilidad ambiental.

Según datos del Eurobarómetro (2007), el 89% de los consumidores europeos consideran que los productos importados desde fuera de la Unión Europea deberían respetar las mismas condiciones de Bienestar animal/ protección aplicadas dentro de la misma.

Los mercados actuales de exportación, en forma creciente utilizan como base la información con garantías de sólida base científica, que certifica la calidad tanto intrínseca como extrínseca del producto. Sin embargo, existe escasa información de estas características a nivel internacional, respecto al Bienestar animal en sistemas de producción extensivos o semiextensivos. En este sentido, la innovación tecnológica en Bienestar animal en países en vías de desarrollo presenta un rezago tecnológico en comparación con los países desarrollados. En Uruguay, la Investigación en estos aspectos es de reciente atención, por lo que nuestro desafío como país exportador, será generar información que permita conocer y demostrar científicamente los atributos y limitantes de los sistemas de producción (del Campo y Montossi, 2007). El avance dispar a nivel nacional y regional, en relación al internacional, se encuentra aún más acentuado en la fase primaria, ya que los países en vías de desarrollo han invertido mayores esfuerzos en el estudio científico de las fases de transporte y prefaena que en los sistemas productivos.

Por otra parte, existen grandes diferencias con los países desarrollados, tanto en los sistemas (extensivos) como en las especies en producción de mayor relevancia (bovinos y ovinos vs. porcinos y aves).

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CAPÍTULO II - REVISIÓN BIBLIOGRÁFICA

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2.1. Calidad de Canal y Carne

La calidad de un producto en sentido genérico, se puede definir como el conjunto de características que le confieren una mayor aceptación y un mayor precio en el mercado. Es complejo de definir cuando se aplica a la carne, debido a la diversidad de factores que están implicados en su manejo, pero en cualquier caso, se puede aceptar que la calidad es la adecuación del producto al uso que se le vaya a dar (Touraille, 1991). Según Sánchez et al. (1998) la calidad es el poder de atracción sobre el comprador y la capacidad para satisfacer a éste cuando se le convierte en consumidor.

2.1.1. Calidad de la Canal

La canal bovina se define como el cuerpo entero del animal sacrificado, sangrado, desollado, eviscerado, separada la cabeza a nivel de la articulación occípito-atloidea y sin extremidades, que se cortarán a nivel de las articulaciones carpo-metacarpiana y tarso-metatarsiana. La canal podrá conservar o no los riñones y la grasa de riñonada y de la cavidad pelviana; carecerá de vísceras torácicas y abdominales, así como de órganos sexuales y sus músculos, de ubre y de grasa mamaria (Reglamento (CEE) del Consejo nº 1208/81). Las características de calidad de las canales se establecen a partir del peso de la canal en caliente, la conformación y el grado de engrasamiento. Dichos parámetros proporcionan información sobre el músculo, la grasa y el hueso en la canal, sobre la cantidad y composición de las piezas, la cantidad de tendones y la constitución de la musculatura y el tejido graso (Schön, 1973).

2.1.1.1. Peso de la canal

El peso de la canal determina su valor comercial, ya que la industria comercia sobre la base de precio por kilo de ésta (Harris, 1982). El peso constituye un indicador de la cantidad de músculo (Tulloh, 1963; Berg y Butterfield, 1966; Robelin et al., 1974) influyendo sobre el tamaño de los cortes que serán producidos y la eficiencia de producción. El aumento del peso de la canal se refleja en un incremento de los espesores musculares y acúmulos adiposos, y por lo tanto de las dimensiones de la misma, así como de todos los componentes que la forman (Sañudo et al., 1997). Un aumento del peso de la canal supone un incremento del tejido adiposo y de las zonas de madurez tardía, una disminución del tejido óseo y de los componentes de desarrollo precoz y una estabilización, más o menos clara, del tejido muscular y de las zonas isométricas, es decir, aquéllas cuyo crecimiento es

proporcional al crecimiento del todo. A peso de canal constante, el nivel de engrasamiento es el que detreminará la variabilidad de los demás componentes de la canal. Según Brito et al. (2007) el peso constituye la medida más simple y precisa de la canal; realizando un aporte altamente significativo en la predicción del peso de corte pistola, cortes valiosos (lomo, bife, cuadril sin tapa, nalgas, peceto y tapa cuadril) y Rump & Loin (de los Campos et al., 2002).

2.1.1.2. Sistemas de clasificación y tipificación de canales

Los sistemas de Clasificación y Tipificación de Canales consisten en la caracterización en forma objetiva y/o subjetiva, de las canales producidas y comercializadas, definiendo la calidad de las mismas al utilizar criterios homogéneos agrupados en distintas categorías según sus características. Dichos sistemas describen el valor de las canales en términos útiles para la industria de la carne. Es un mecanismo de comunicación entre productores, industriales y consumidores.

El objetivo final de estos sistemas es:

- a) anticipar y satisfacer las exigencias de los mercados (doméstico y de exportación) tanto en calidad como en cantidad y uniformidad
- b) generar información para incrementar el valor agregado del producto final y la eficiencia del sistema como un todo, permitiendo retroalimentar los diferentes eslabones de la cadena (Brito et al., 2007).

El método más extendido para la valoración de la canal es la apreciación visual de los perfiles de las diferentes regiones anatómicas y el grado de engrasamiento, mediante comparación con patrones fotográficos (Kempster et al., 1982) utilizando escalas de puntuación variable. En los últimos años, INIA Uruguay en conjunto con INAC y contando con la participación de un conjunto de establecimientos frigoríficos, ha estado realizando las primeras pruebas de validación de un sistema de valoración objetiva de carcassas, incluyendo técnicas de análisis de imágenes (VIASCAN, BEEFCAM). Con ello se intentará dar una base más objetiva y consistente al proceso de tipificación de reses. Dicho proyecto se ha desarrollado en el marco de un convenio con la Universidad de Colorado (USA) (Ordeix y Ferreira, 2001).

2.1.1.2.1. Conformación

La Comisión de Estudio sobre Producción Bovina de la Asociación Europea para Producción Animal, define a la conformación como el espesor de los planos musculares y adiposos en relación al tamaño del esqueleto (De Boer et al., 1974). Su evaluación pretende medir la cantidad de carne vendible o consumible, especialmente de sus partes más selectas

(Sañudo et al., 1997). El método más reciente y generalizado para determinar la conformación, consiste en establecer una escala creciente de clases, tomando como referencia modelos fotográficos como el que propone el sistema comunitario en vigor, R. (CEE) nº 1208/81, R. (CEE) nº 2930/81 y R.(CEE) nº 1026/91, así como el utilizado en Uruguay, propuesto por el Instituto Nacional de Carnes (INAC, 1997).

2.1.1.2.2. Engrasamiento

El estado de engrasamiento se define como la proporción de grasa que presentan las canales respecto de su peso. Es uno de los factores que producen mayor variación en el valor comercial de una canal (Briskey y Bray, 1964). El estado óptimo de engrasamiento es el que compagina la cantidad mínima de grasa para satisfacer los gustos del consumidor, con la cantidad suficiente para asegurar las condiciones de jugosidad de la carne, de presentación y de conservación de la canal (Ruiz de Huidobro et al., 1996). La determinación del engrasamiento se suele realizar mediante la calificación del estado de engrasamiento y la apreciación visual de la cantidad de grasa presente en la cara interna de la cavidad torácica y en el acúmulo pélvicorenal. Este engrasamiento ejerce una notable influencia en la cantidad de carne vendible entre canales de peso semejante (Ramsey et al., 1963; Cuthbertson , 1979) y por este motivo debe de ser considerado por el productor para tratar de adaptar su producción a los requerimientos del mercado.

2.1.1.2.3. Sistema de clasificación y tipificación bovina utilizado en Uruguay

El Sistema Oficial de Calificación y Tipificación de Carnes Vacunas de Uruguay (INAC, 1997), agrupa a los animales en función de edad y sexo. En cuanto a Tipificación, define conformación (INACUR) y terminación (0 al 4), donde “I” es mejor conformación que “R” y “4” es mayor engrasamiento que “0”

Conformación

- I. Tienen un gran desarrollo muscular en todas sus regiones anatómicas. Reses cilíndricas, largas, compactas. Líneas convexas.
- N. No son reses tan compactas como las anteriores. Líneas de menor convexidad.
 - A. Es el grupo más abundante en Uruguay (Novillos). Relación carne/hueso equilibrada. Líneas algo deprimidas.
 - C. Líneas rectas, reses ligeramente descarnadas, predominando el cuarto delantero.
 - U. Conformación deficiente con líneas angulosas y poco desarrollo muscular.
 - R. Hay carencia muscular y sus contornos son deprimidos.

Terminación

0. Cobertura muy escasa o carencia total. Son llamadas canales magras.
1. Escasa cobertura, grandes áreas sin cubrir (pierna y cogote).
2. Grasa moderadamente abundante. Distribución uniforme.
3. Cobertura grasa abundante y uniforme. Se aceptan excesos en grupa, parrilla costal y escapular.
4. Grasa de cobertura excesiva apareciendo fláccida y con aspecto grumoso.

2.1.1.2.4. Sistema de clasificación y tipificación bovina utilizado en la Unión Europea

El Consejo de La Unión Europea considera que la clasificación de las canales de vacuno pesado debe llevarse a cabo basándose en la conformación y en el estado de engrasamiento (Reglamento CE nº 1183/2006). La utilización combinada de estos dos criterios permite distribuir las canales en clases. La clasificación de las canales de vacuno pesado se efectuará valorando sucesivamente:

- a) conformación (SEUROP, donde S es mejor conformación que P)
- b) grado de engorde (1 a 5, donde 5 es mayor engrasamiento que 1).

Conformación

- S.** Superior. Todos los perfiles extremadamente convexos; desarrollo muscular excepcional con dobles músculos (tipo “culón”).
- E.** Excelente. Todos los perfiles de convexos a superconvexos; desarrollo muscular excepcional.
- U.** Muy buena. Perfiles convexos en conjunto; fuerte desarrollo muscular
- R.** Buena. Perfiles rectilíneos en conjunto; buen desarrollo muscular.
- O.** Menos buena. Perfiles rectilíneos a cóncavos; desarrollo muscular medio.
- P.** Mediocre. Todos los perfiles de cóncavos a muy cóncavos; escaso desarrollo muscular.

Terminación

1. No graso. Cobertura de grasa inexistente o muy débil.
2. Poco cubierto. Ligera cobertura de grasa, músculos casi siempre aparentes.

3. Cubierto. Músculos, excepto cadera y paletilla, casi siempre cubiertos, escasos acúmulos de grasa en el interior de la cavidad torácica.
4. Graso. Músculos cubiertos de grasa, pero aún parcialmente visibles a nivel de la cadera y de la paletilla, algunos acúmulos pronunciados de grasa en el interior de la cavidad torácica.
5. Muy graso. Toda la canal cubierta de grasa, acúmulos importantes de grasa en el interior de la cavidad torácica.

2.1.1.3. Composición de tejidos de la canal

La valoración cuantitativa de una canal comprende la evaluación de los tres tejidos de interés productivo que la componen (óseo, muscular y adiposo), determinando la cantidad y la proporción en la que se encuentran. La relación entre estos parámetros constituye el determinante casi exclusivo del valor económico del animal en cualquier mercado (Ruiz de Huidobro et al., 1996). La grasa es el componente de la canal que presenta mayor variabilidad en el aspecto cuantitativo y cualitativo (Briskey y Bray, 1964) y condiciona la proporción relativa de los otros dos componentes (Berg y Butterfield, 1976). El hueso, al constituir el tejido más consistente y menos variable, se considera normalmente como residual, sin embargo posee una importancia decisiva en la composición de los animales, dado por la relación músculo/ hueso.

Conforme aumentan la edad y el peso del animal, el porcentaje de grasa en la canal aumenta proporcionalmente, el porcentaje de hueso disminuye y el de músculo se mantiene constante (Sañudo et al., 1997). Según Robelin et al. (1986) la canal ideal es aquella que tiene un alto porcentaje de tejido muscular, una cantidad suficiente de grasa infiltrada y una proporción de grasa de cobertura limitada. Óptimos niveles de grasa son importantes ya que las canales requieren un mínimo de grasa subcutánea necesaria para minimizar las pérdidas de humedad de la canal tras el sacrificio así como para protegerla de la desecación, las quemaduras por frío del músculo y la contaminación bacteriana en cámara frigorífica (Cuthbertson y Kempster, 1979; Delfa, 1994). La grasa de cobertura además protege a las fibras musculares del fenómeno del acortamiento del frío o “cold shortening” (Smith et al., 1976). Tanto la cantidad como la composición de la grasa, puede variar en función de la especie animal, la edad, el sexo, el régimen alimenticio, la localización anatómica y el entorno medioambiental (Kempster, 1981).

El tejido adiposo de la canal está constituido por cuatro tipos de grasa: la interna (renal y pélvica), la intermuscular, la subcutánea y la intramuscular. Según Robelin et al. (1974) el orden cronológico de deposición de la grasa establecido para los bovinos es: intermuscular, interna, subcutánea, y por último, la intramuscular.

El contenido de grasa intramuscular se mide de manera subjetiva a nivel del área del ojo de bife, mediante el uso de escalas de grados (USDA, 1997) que va desde D (desprovisto de grasa) hasta A (abundante); pasando por Pd (prácticamente desprovisto), Tr (trazas), SI (leve), Sm (Poco), Mt (modesto) Md (moderado), SIA (levemente abundante), Mda (moderadamente abundante).

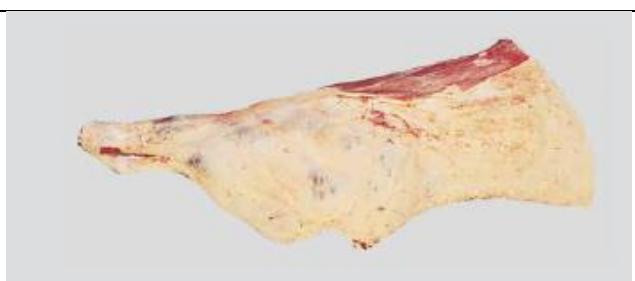
2.1.1.4. Color de la grasa

El color de la grasa presenta un efecto importante en las preferencias del consumidor (Barton, 1970). La terminación de animales en base a pasturas incrementa los valores del b^* o amarillamiento de la grasa respecto a animales alimentados en base a concentrados (Bennett et al., 1995; Realini et al., 2004; Schaake et al., 1993; Simonne et al., 1996). Esto es debido a las importantes concentraciones de β caroteno en las pasturas, en comparación con los granos (Tume y Yang, 1996). La cantidad de β caroteno en plasma, músculo y tejido adiposo puede incrementar hasta en un 50 %, a medida que los animales pasan mayor tiempo en pasturas (Yang et al., 2002). La terminación de animales en base a pasturas generalmente está asociada a un mayor período de engorde, por lo cual los animales se faenan con más edad y ello también puede contribuir a un mayor amarillamiento de la grasa (Shemeis et al., 1994).

2.1.1.5. Cortes valiosos

Desde el punto de vista anatómico, de la composición de la canal resultan una serie de cortes primarios (Tabla 1) y a partir de ellos, una serie de cortes comerciales que se clasifican en distintas categorías en función de su terneza potencial. El corte pistola representa más del 40% de la media canal y más del 80% del cuarto trasero. Un rendimiento elevado de este corte indica una buena conformación de las regiones dorso lumbar, de la grupa y del muslo, zonas donde se asientan los cortes de mayor valor. Los cortes que surgen de la pistola son lomo, bife angosto, cuadril, nalga de adentro, nalga de afuera, bola de lomo, colita de cuadril, tortuguita y garrón (Robaina, 2002). El bife, cuadril y lomo ("Rump and Loin") constituye entre el 7 % y el 10 % del peso de la canal (Franco et al., 2002). A las piezas de mayor terneza como es el caso del lomo o solomillo, les corresponde una categoría extra. En el caso de países exportadores como Uruguay, los cortes sin hueso se definen en función del mercado a que se destine el producto. En trabajos de Investigación se manejan los estándares de Alemania o Inglaterra ya que son los más utilizados para los destinos de exportación actuales. Estos estándares consisten en 7 u 8 cortes valiosos (dependiendo del mercado) sin hueso, del trasero (Figura 1). El producto neto comestible para el consumidor incluye el músculo sin hueso y con el recorte de grasa necesario (grasa separable).

Tabla 1. Media canal bovina y cortes primarios con hueso. Fuente: INAC, 2007.

Media canal	Cuarto trasero	Pistola	Espinazo con cuadril (Rump y Loin)
Se obtiene a partir de la canal, mediante un corte sagital (al eje de la columna vertebral), que divide en dos, los cuerpos vertebrales	Porción caudal de la Media canal que resulta de seccionarla mediante un corte transversal a la columna vertebral, siguiendo el espacio intercostal entre la 10 ^a y la 11 ^a costilla	Corte preparado a partir del Cuarto trasero una vez extraídos el flanco abdominal (Vacío), el Asado y las porciones de Falda y Pecho cuando corresponda. Se obtiene mediante un corte que comienza en el pliegue de la babilia a nivel del ganglio pectoral, se dirige a medial bordeando los músculos de la pierna y continúa paralelo a la columna vertebral bordeando el bife angosto	
			
			
			
			

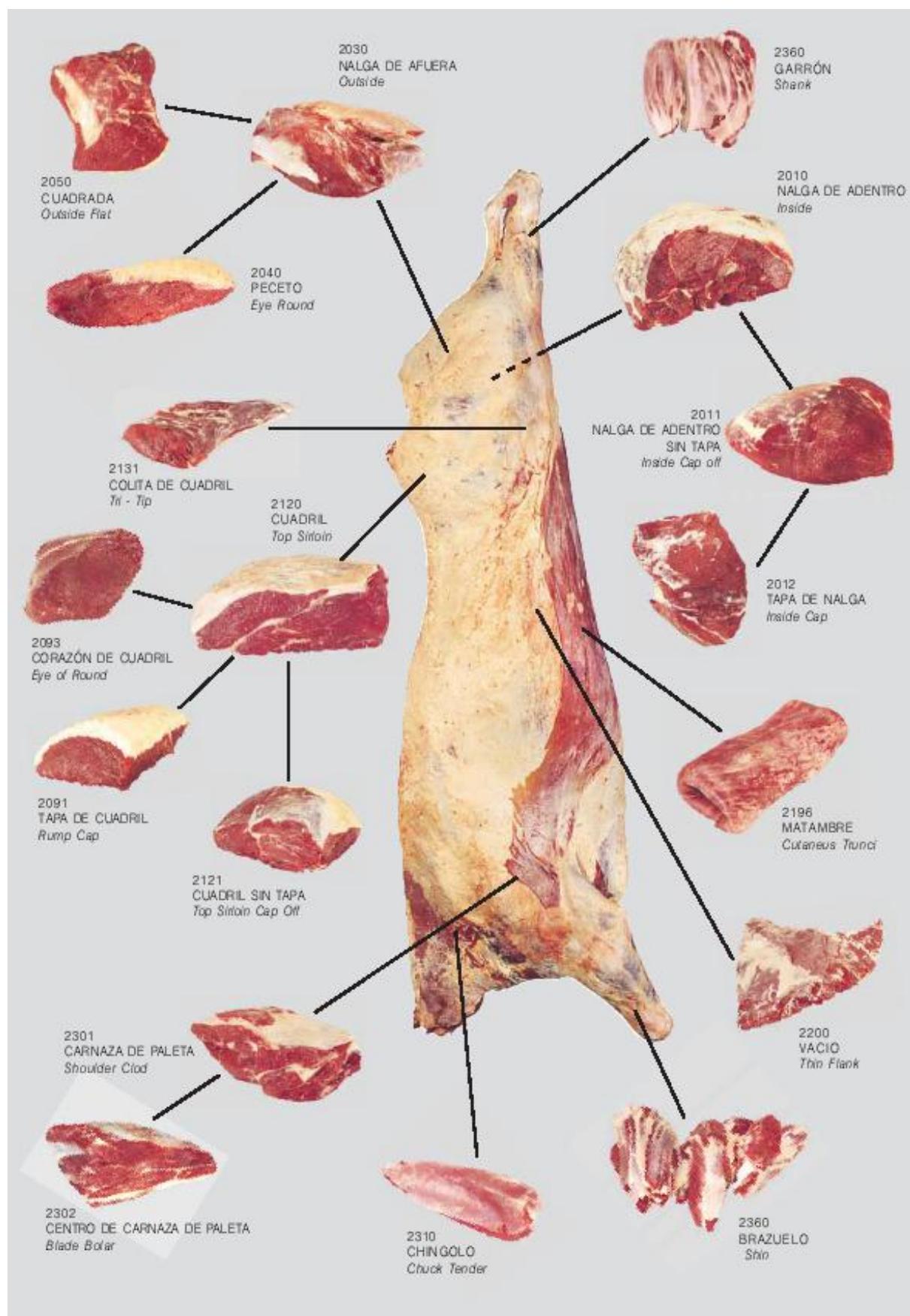


Figura 1. Cortes bovinos sin hueso.

Fuente: INAC, 2007.

2.1.1.6. Factores que afectan la calidad de la canal

En la siguiente Tabla se resumen los principales factores que podrían influir sobre los parámetros relacionados con la calidad de la canal.

Tabla 2. Factores que influyen en parámetros relacionados con la calidad de la canal.

Calidad de la canal			
	Peso	Conformación	Engrasamiento
Factores intrínsecos			
Raza	***	****	***
Genotipo	**	****	**
Sexo	***	**	***
Edad-peso	****	*	****
Factores productivos y medioambientales			
Ambiente-Estación	***	0	**
Alimentación	***	*	****
Factores de sacrificio y presacrificio			
Transporte-Estrés-Ayuno	*	0	0
Sacrificio	**	0	*
Postsacrificio y comercialización			
Maduración	0	0	0
Refrigeración de las canales	*	0	0
Conservación	0	0	0

0: sin influencia; *: pequeña infl.; **: infl. moderada; ***: infl. alta; ****: infl. muy alta

Fuente: adaptado de Sañudo (1993).

La alimentación que reciben los animales influye sobre el peso, la edad a la faena, el grado de terminación del animal y sobre la composición de la res. Los efectos del nivel de nutrición se deben al nivel de ingesta energética en relación con las necesidades de crecimiento proteico. Esto implica la existencia de un límite biológico en el potencial fisiológico de un animal para depositar proteína, almacenando el resto de la energía consumida, como grasa (Gorrachategui, 1997).

Las razas de madurez tardía o alto potencial de crecimiento (Nelore, Salers, Braford) tienden a presentar pesos de faena y pesos de canal superiores a las razas británicas (Angus y Hereford) cuando se utiliza el mismo grado de terminación como criterio de faena (Franco et al., 2002). Generalmente, al ganado vacuno precoz tiene un tamaño más pequeño y entra en la fase de engrasamiento a pesos más bajos (Berg y Butterfield, 1979), es por ello que las razas británicas (Hereford y Angus) comienzan a engrasarse a un peso menor que las

razas continentales (Brito et al., 2003), mientras las razas de maduración tardía continúan depositando músculo, haciendo variar de esta forma las relaciones músculo/hueso/grasa.

Respecto al manejo pre faena, se destaca que el estrés sufrido por los animales en las fases previas al sacrificio puede presentar un efecto muy importante sobre la calidad de la canal. Varios autores han reportado un incremento en la incidencia de hematomas en animales que han sufrido un manejo inadecuado (Gregory, 1996, 2003). Esto implica la remoción de tejidos de la zona afectada con un posible efecto sobre el peso y el rendimiento de la canal. Algunos autores sostienen que más del 50 % de los hematomas ocurren en la planta de faena (McCausland y Millar, 1982). Sin embargo, el manejo pre embarque y el transporte también son muy importantes en este sentido.

Durante el transporte y la industrialización pueden producirse otras pérdidas en la canal que se agruparían de la siguiente manera (Robaina, 2002):

- ❖ Mermas por desbaste: pérdidas de materia fecal, orina y evaporación a nivel de piel en un período dado. Implica una disminución del peso vivo sin influenciar el peso de la canal (Robaina y Castro, 2003)
- ❖ Pérdidas de proceso (producidas principalmente en la faena): si se practican períodos muy extensos de transporte y ayuno, se agrega la merma por pérdida de tejidos que se produce fundamentalmente vía evaporación de agua a través de los pulmones. Esta deshidratación produce pérdidas de peso de los tejidos que luego formaran parte de la canal. (Robaina y Castro, 2003)
- ❖ Pérdidas de enfriado: Se produce particularmente por acción de la circulación forzada del aire frío. Las medias canales sufren una merma en su peso que se calcula entre un 1.8 y 2.2 %. También puede producirse un deterioro en su calidad por la deshidratación superficial (aspecto seco y oscuro de la carne en las zonas donde no está protegida por la cobertura de grasa).

Durante las 24-48 horas de ayuno pre faena, la mayoría de las pérdidas de peso son debidas a la excreción del contenido del tracto gastrointestinal y de orina. Cuando el ayuno excede las 48 horas, (alimento y agua), comienza el catabolismo de tejidos y la deshidratación, contribuyendo de esta forma a las pérdidas de peso no deseadas (Ferguson y Warner, 2008).

2.1.2. Calidad de carne

No existe una clara conexión o interrelación entre la calidad de la canal y la calidad de la carne. El actual sistema de valoración visual de las canales es insuficiente y no permite emitir juicios precisos sobre índices de calidad de carne (Kirton, 1989). Las características o factores de calidad de la carne pueden agruparse en cinco grandes grupos:

- 1- Factores bioquímicos (pH, capacidad de retención de agua, colágeno, estado y consistencia de la grasa, estado de las proteínas, viscosidad, estabilidad oxidativa)
- 2- Factores sensoriales u organolépticos (color, veteado, exudado, dureza, jugosidad, sabor y olor)
- 3- Factores nutricionales (valor proteico, aminoácidos esenciales, grasa, composición en ácidos grasos, vitaminas y minerales)
- 4- Factores higiénicos y toxicológicos (como garantía de no producir un riesgo para la salud del consumidor)
- 5- Factores de calidad social (como garantía de que la carne ha sido producida considerando el Bienestar animal y el medio ambiente).

2.1.2.1. Factores bioquímicos o tecnológicos

2.1.2.1.1. Tasa de descenso del pH y la temperatura

Desde el punto de vista de las características tecnológicas de la carne, tiene tanta importancia el valor del pH final, como la tasa de descenso del mismo. Esta depende de factores intrínsecos tales como la especie, el tipo de músculo, el temperamento y la variabilidad entre animales, así como de factores extrínsecos tales como la temperatura ambiente, el manejo y la alimentación pre faena que influyen sobre las reservas de glucógeno del músculo en el momento del sacrificio (Immonen et al., 2000; Sañudo, 1992). La relación entre el descenso de la temperatura y el pH de la canal desde el momento de la faena hasta alcanzar el pH final, es de gran importancia. El descenso de ambas variables deberá estar dentro de lo que se denomina la ventana ideal. Dicha “ventana” requiere que el pH sea mayor a 6 mientras la temperatura está por encima de 35° C y menor a 6 antes de que la temperatura descienda por debajo de 12 - 15° C (Figura 2). Si la temperatura desciende a 12 grados y el pH permanece alto, ocurre el llamado acortamiento por frío (cold shortening). Si por el contrario, la temperatura de la canal es alta y el pH desciende muy rápidamente (Figura 2), ocurre acortamiento por calor (heat shortening).

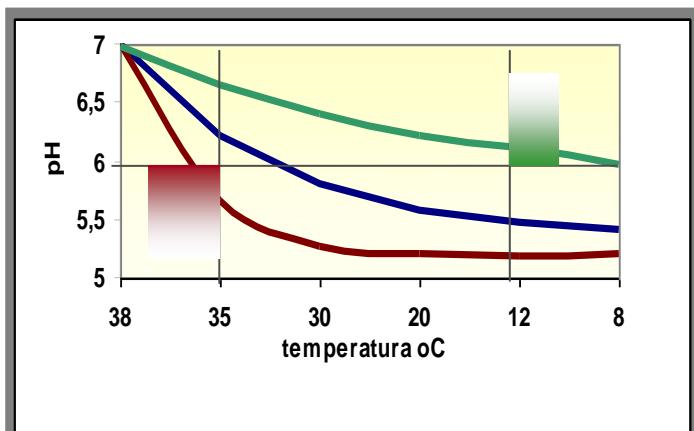


Figura 2. Curvas de descenso de pH y temperatura muscular (Meat Standard Australia - MSA). Fuente: Thompson, 2002.

La duración de esta etapa (descenso de pH) es de aproximadamente 24 horas postsacrificio en vacuno, momento a partir del cual se estabiliza el pH y comienza la etapa de maduración.

Factores que afectan el descenso del pH

Entre los diversos factores que afectan el descenso del pH de la canal, se destaca a la alimentación, el manejo y el temperamento de los animales, como los más relevantes para el presente trabajo de tesis.

Según Pethick y Rowe (1996) los principales motivos de falta de glucógeno en el músculo previo al sacrificio, son el mal manejo durante el transporte y en planta frigorífica, así como un inadecuado plano de nutrición en la etapa de terminación.

En bovinos, dietas con altos niveles de energía como las ofrecidas en condiciones de engorde a corral, permiten incrementar las reservas de glucógeno en el músculo y de esta manera se lograrían adecuados descensos de pH. De igual forma, la suplementación con granos durante la etapa de terminación incrementa las reservas de glucógeno permitiendo una correcta acidificación y por lo tanto una adecuada conservación de la carne (Immonen et al., 2000). Las reservas de glucógeno podrían realizar un efecto “buffer” en el período pre faena (Immonen et al., 2000). Diversos autores han encontrado una tasa de descenso de pH más rápido en animales alimentados con dietas ricas en energía, respecto a canales de animales alimentados en base a pasturas (Espejo et al., 1998; French et al., 2001). El pH final obtenido en canales de animales de pasturas es mayor (Muir et al., 1998), aunque no necesariamente implica problemas de calidad. Por otra parte, se considera que la mayor

cobertura de grasa que presentan los animales alimentados con concentrado (Bennet, 1988; Keane y O'Ferrall, 1992; Korver et al., 1987; Micol, 1993), podría traducirse en un enfriamiento más lento de la canal (Mooney et al., 1998). Las canales más engrasadas permitirían un descenso más lento de la temperatura *post mortem* y por tanto una tasa de descenso de pH más adecuada. Sin embargo, estas propiedades pueden verse disminuidas si, tal como se ha mencionado antes, el manejo de los animales en los momentos previos a la faena compromete las reservas de glucógeno del músculo. Otros autores no encuentran relación entre el tipo de alimentación y el pH final (French et al., 2000, 2001; Morris et al., 1997). La controversia de los resultados que relacionan el pH y la alimentación, podría estar explicada por diversos factores de confusión, tales como las diferencias raciales, diferencias individuales en respuesta al estrés, características y condiciones del transporte y manejo pre faena.

En aquellos animales que llegan muy fatigados al momento de la faena, el pH desciende escasamente y en forma muy lenta, debido a que el glucógeno del músculo se ha consumido antes del sacrificio (Sañudo, 1992). No solo el rigor mortis se instalará antes en aquellos animales que no presentan reservas de glucógeno y energía (stress) sino que la poca disponibilidad de sustrato glicolítico en el músculo, no permitirá la correcta acidificación del mismo. Un pH último elevado en el músculo bovino, puede causar el indeseable fenómeno de corte oscuro (Kidwell, 1952). Además de la apariencia desagradable de este fenómeno, se ve facilitado el crecimiento bacteriano (Lawrie, 1998).

Además del manejo y de la alimentación previa a la faena, el temperamento de los animales es otro factor importante que debe considerarse al hacer referencia a calidad de producto. Animales bien alimentados y con períodos de descanso adecuados previo a la faena (lo cual indicaba que tendrían cantidades de glucógeno adecuadas en el músculo), mostraron carne con valores elevados de pH final (Howard y Lawrie, 1956). Estos animales presentaban temperamentos excitables, y a pesar de que las tensiones sufridas no se reflejaban en movimientos físicos, se reducían las reservas de glucógeno del músculo, ocasionando por tanto, mayores valores de pH (Petaja, 1983).

2.1.2.1.3. Capacidad de retención de agua (CRA)

La CRA es la capacidad de la carne para retener total o parcialmente el agua propia o la adicionada durante su tratamiento (Hamm, 1960). El contenido en agua y su distribución tiene gran influencia en las propiedades de la carne, especialmente en características nutritivas (por pérdidas de minerales y todos aquellos componentes solubilizados como proteínas, vitaminas, etc.) y sensoriales (dureza, jugosidad, color y apariencia) (Davey y Gilbert, 1974; Offer et al., 1989). Por otra parte presenta una gran relevancia económica, ya

que al perder agua, se pierde peso. La mayoría del agua del músculo se encuentra entre los espacios de los filamentos finos y gruesos de las miofibrillas. Todos los factores que afectan el estado de entramado de los filamentos de las miofibrillas, tales como el grado e intensidad de la acidificación *post mortem* del músculo, también afectan a la cantidad de exudado perdido por la carne (Warris, 2003). Según dicho autor, una acidificación reducida y un pH final elevado, ocasionan bajas pérdidas por exudado (es el caso de carnes DFD), mientras que un grado de acidificación inicial alto conduce a mayores pérdidas por exudado. Algunos autores han reportado mayores pérdidas por cocción en la carne proveniente de animales alimentados a pasturas (Mandell et al., 1998; Vestergaard et al., 2000). En este sentido, García Torres et al. (1998) observan en terneros de raza Retinta, una mayor CRA en aquellos que fueron alimentados en base a concentrado. Por el contrario, Alberti y Sañudo (1987), observaron que el largo período de terminación en base a pasturas, incrementó la CRA, mientras que la naturaleza del pasto empleado no influyó sobre ésta. Otros autores no han encontrado diferencias en pérdidas por cocción, al comparar carne proveniente de diferentes estrategias de alimentación (Cerdeño et al., 2006; French et al., 2001; Kerth et al., 2007). Según Wismer-Pedersen, (1994), la capacidad de retención de agua disminuye con la edad en bovinos. Este mismo efecto ha sido observado por otros autores en el ganado ovino (López, 1987; Sañudo y Sierra, 1982).

2.1.2.2. Factores sensoriales u organolépticos

2.1.2.2.1. Color de la carne

El color es uno de los atributos sensoriales más importante en el momento de decidir la compra por parte del consumidor (Krammer, 1994; Shackelford et al., 1991). El consumidor en general prefiere carne de color rojo brillante, mientras que rechaza la de color apagado o pardo (Beriain y Lizaso, 1997). No obstante, en la aceptación del color influyen factores geográficos, sociales y culturales. Desde un punto de vista físico, el color de la carne es el resultado de la distribución espectral de la luz que la ilumina, y de la intensidad de la luz reflejada por su superficie. Se considera como una característica tridimensional de los objetos, determinada por un atributo de claridad y dos atributos cromáticos, el tono y la saturación (Asenjo, 1999). El color de la carne se evalúa a través del sistema CIELAB, que considera a las coordenadas L* (luminosidad), a* (índice de rojos) y b* (índice de amarillos).

Varios autores mencionan que la luminosidad de la carne depende de diversos factores tales como el pH, la capacidad de retención de agua, la integridad de la estructura muscular, y en menor medida del grado de oxidación de los hemopigmentos y de la grasa. Palombo y Wijngaards (1990); Pérez-Alvárez et al. (1998) y Sayas (1997) reportaron que el contenido en grasa es otro factor a tener en cuenta sobre esta coordenada, pues las materias primas

con mayor contenido en grasa, son las que presentan mayores valores de L^* . Estos resultados parecen indicar la existencia de una posible relación inversa entre la concentración de mioglobina de la carne y la coordenada L^* a valores de pH similares.

La coordenada a^* (rojo-verde) está relacionada con el contenido de mioglobina (Pérez-Alvárez et al.; 1998). Kang et al. (1998) sostienen que el valor de a^* debería ser usado como un estimador de la concentración de mioglobina y para predecir el color de la carne. Si bien el color de la carne estaría determinado por la concentración de mioglobina que presenta, el estado de óxido-reducción de la misma también sería determinante de dicha coloración. De hecho, el valor de la coordenada b^* (amarillo-azul) ha sido relacionado con los distintos estados de la mioglobina (Pérez-Alvárez, 1996).

Algunos autores argumentan que el color de la carne depende del contenido y estado de la mioglobina (principal pigmento de la carne) así como de la estructura de la superficie y de la proporción de grasa intramuscular (Judge et al., 1989; Renerre, 1981).

Factores que afectan el color de la carne

Según Honikel (1998) existen tres fuentes en la variación del color de la carne. La primera, de tipo intrínseco, es el contenido en pigmentos del músculo, el cual depende de factores de producción tales como la especie, la edad y régimen nutricional. La segunda fuente se refiere a las condiciones de manejo en los períodos presacrificio, sacrificio y postsacrificio, por la influencia en el pH y en la temperatura. La tercera está relacionada con el tiempo de almacenamiento y con los procesos de oxigenación y oxidación. Todos aquellos factores que afectan a las propiedades ópticas de la carne, pueden tener una influencia significativa en el color (pH, capacidad de retención de agua, veteado, tejido conectivo, tamaño de las fibras musculares y la desnaturalización de las proteínas (MacDougall, 1982).

Entre los diferentes factores que afectan el color de la carne, se destaca a la alimentación, la edad de los animales y el manejo prefaena, como los más relevantes desde el punto de vista del presente trabajo de tesis.

Varios autores han reportado que la carne de terneros alimentados con pasturas, presenta una coloración más oscura que la de aquellos terminados con concentrados (Bidner et al., 1986; Crouse et al., 1984). El incremento en la grasa intramuscular en bovinos, que ocurre cuando se suministran granos, asociado al color blanco le imprimiría cierta claridad a la carne, distinta de la proveniente de sistemas pastoriles (Priolo et al., 2001). Bennet et al. (1995) reportaron mayores valores de a^* en carne de animales provenientes de pasturas al comprarlos con los de concentrado, y lo atribuyeron a la mayor edad de los primeros al momento de la faena. Por otra parte, algunos autores no encontraron diferencias en el color

de la carne de animales terminados en base a concentrados y a pasturas (Alberti y Sañudo, 1987; Alberti et al., 1992; Cerdeño et al., 2006; French et al., 2001). Es por ello que varios autores sostienen que el efecto de la naturaleza de la alimentación en rumiantes no reviste capital importancia sobre las características cromáticas de la carne (Alberti et al., 1992; Hedrick et al., 1983), posiblemente como consecuencia de los procesos de transformación que tienen lugar en el rumen. La actividad que desarrollan los diferentes músculos también estaría influyendo sobre la coloración de los mismos. Según Lawrie (1998) aquellos músculos que están desarrollando una actividad mayor presentan una coloración más oscura que aquellos que se ven sometidos a una menor actividad. La mioglobina ejerce funciones de almacenamiento y transporte de oxígeno necesario para el músculo, por lo que su concentración aumenta a medida que crece la demanda de oxígeno. Es por ello que es superior en los músculos más activos y en los animales de mayor edad (Cepero y Sañudo, 1996), ambas condiciones relacionadas a los sistemas extensivos de producción. .

Los animales más viejos presentan mayor cantidad de mioglobina que los jóvenes, dando un color más oscuro a la carne (Bruwer et al., 1987; Cross et al., 1984; Lawrie, 1977; Morbidini et al., 1994). En este sentido, Gil et al. (1998) reportaron una correlación positiva entre el contenido en pigmentos y la edad del animal. Por otro lado, con la edad aumenta el estado de engrasamiento y disminuye la permeabilidad capilar, lo cual dificulta la transferencia de oxígeno hasta la fibra muscular y por ello es necesaria una mayor cantidad de mioglobina muscular para garantizar el aporte de oxígeno adecuado (Renerre y Valin, 1979). Boccard (1986) reportó que a mayores pesos de canal, la carne presentaba un color más rojo (mayores valores de a^*). Dentro de un mismo animal, existe gran variabilidad en el contenido en pigmentos entre los distintos músculos, en función de la composición de fibras rojas, ricas en mioglobina, o fibras blancas, pobres en mioglobina (Cassens, 1977; Renerre, 1981).

El manejo de los animales en los períodos previos al sacrificio influye sobre el color, a través de su efecto sobre el pH de la canal. El estrés sufrido en los momentos previos a la faena, podría reducir el glucógeno del músculo *in vivo* (Tarrant, 1988; Warris, 1990) no permitiendo el correcto descenso del pH. Normalmente, cuando la carne fresca es cortada, cambia del color púrpura al rojo brillante (proceso conocido como blooming). Cuando la carne tiene altos valores de pH último debido a un mal manejo, no ocurre este proceso y las carnes permanecen oscuras. Los altos niveles de pH y por tanto la elevada capacidad de retención de agua entre las cadenas proteicas, hace que las fibras se hinchen y la superficie de la carne refleje una menor cantidad de luz (Renerre, 1988). Cuando el pH desciende a valores cercanos al punto isoeléctrico de las proteínas, se pierde capacidad de retención de agua, las cadenas de proteína se unen dando lugar a una estructura que impide que la luz penetre

fácilmente y es reflejada, dando lugar a un color más claro. Según Page et al. (2001) el pH de la carne presenta una correlación negativa con el color (valores de rojos y amarillos). Altos valores de pH final implican carnes con menores valores de a^* y $*b$, mientras que bajos valores de pH final, estarían relacionados a un color de la carne más rojo (mayor $*a$) y más amarillo (mayor $*b$).

2.1.2.2.3. Terneza

La terneza y el color de la carne son los parámetros principales que determinan las preferencias del consumidor (Pearson, 1966). Para Dransfield et al. (1984) y Seideman et al. (1989) la terneza es el parámetro más importante de la calidad sensorial de la carne desde el punto de vista de los consumidores, siendo una cualidad sensorial especialmente importante en el ganado vacuno (Sañudo, 1993). Según Brito y Pittaluga (2003) es la característica que determina la aceptación del producto por parte del consumidor y es determinante en la repetición de la compra. La terneza es un atributo muy complejo en el cual participan factores inherentes al animal y al manejo pre y post faena, así como también la forma de preparación del producto. Según Van Hoof (1981) la terneza está determinada por el grano de la carne y el tipo de fibras musculares (tamaño de haces de fibras y número de fibras de cada uno contiene), la longitud del sarcómero y las miofibrillas, y por último, la cantidad y naturaleza del tejido conjuntivo, particularmente la fracción del colágeno. Con esto último coincide Tornberg (1996) quien agrega como factor determinante, a la riqueza en grasa infiltrada. Warris (1990) menciona que la intensidad de los cambios proteolíticos que ocurren en el período de acondicionamiento post mortem, es determinante en la terneza de la carne. En ese sentido, según Dransfield (1977) la textura de la carne depende de la contribución relativa de las proteínas miofibrilares y del tejido conectivo intramuscular, así como del colágeno soluble (Bailey y Lawson, 1989; Gerrard et al., 1987). Las proteínas sarcoplasmáticas son solubles en agua por lo que podría pensarse que su contribución a la terneza no sería de gran relevancia. Sin embargo, algunos autores sostienen que ésta no debe subestimarse (Clarke et al., 1980; Scopes, 1970).

Durante las 24 a 36 horas luego de la muerte del animal, el fenómeno predominante es la glicólisis. Sin embargo, otros procesos degradativos han comenzado. La baja disponibilidad de energía luego de la muerte del animal, además de todos los efectos que ocasiona, incrementa la dificultad para mantener la estructura integral de las proteínas. El bajo pH y la acumulación de ácido láctico facilitan la desnaturalización de las proteínas y ésta es acompañada por una pérdida en la capacidad de retención de agua. A medida que desciende el pH, las proteínas miofibrilares se acercan a su punto isoeléctrico. La desnaturalización puede ser definida como un reacomodamiento físico o molecular de las proteínas que no incluye la hidrólisis de los puentes químicos que unen los aminoácidos

constitutivos de las cadenas polipeptídicas (Putnam, 1953). Va acompañada de un incremento de la reactividad de varios grupos químicos, una pérdida de actividad biológica (enzimas y hormonas), cambios en la forma o tamaño de las moléculas y un descenso de la solubilidad. La desnaturación de las proteínas sarcoplasmáticas las hace vulnerables al ataque de las proteasas del músculo (De Duve y Beaufay, 1959). Es así que la maduración está asociada a incrementos en la terneza de la carne debido a la desnaturación y proteólisis de las proteínas miofibrilares (no incluyendo la dissociación de la actina y la miosina) y las proteínas sarcoplasmáticas. Respecto a las proteínas del tejido conectivo, Sharp (1959) sostiene que estas no sufren proteólisis. Sin embargo, debido a la acción de ciertas enzimas lisosomales, los enlaces en las moléculas de colágeno parecen debilitarse (Etherington, 1971).

Actualmente existe un consenso en que el proceso de maduración es de naturaleza enzimática. Los sistemas proteolíticos más estudiados han sido el de las catepsinas, el de las calpaínas y más recientemente el complejo proteosoma (20S) (Santandreu et al., 2002). Parte de los cambios ocurridos en las proteínas miofibrilares durante la maduración, se deben aparentemente a liberación masiva de iones de calcio desde el retículo sarcoplasmático, el cual pierde la capacidad de retenerlo luego de la muerte del animal. La terneza incrementa debido mayoritariamente a la estimulación que presentan las calpaínas en la presencia de esos iones de calcio. Otros autores sostienen que iones de calcio *per se*, podrían provocar cambios en ciertas proteínas miofibrilares (Taylor y Etherington, 1991). Las calpaínas son inhibidas por una tercera proteína, la calpastatina (Lawrie, 1998). A valores de pH de 6, las calpaínas y calpastatinas se encuentran unidas, pero esta acción inhibitoria cesa cuando el pH cae desde 6 hasta 5.5. Ante estas nuevas condiciones de pH, las calpaínas activas degradan, proteolizan a su propio inhibidor (Lawrie, 1998). Como se ha dicho antes, las calpaínas no actúan sobre las uniones de la actina y la miosina. El 50 % de los cambios ocasionados por dichas enzimas ocurre en las primeras 12 horas post mortem, cuando la temperatura desciende desde 37 a 10 grados centígrados. El pH óptimo de las mismas es por encima de 6. Esto estaría indicando que el incremento de la terneza puede comenzar antes de que se alcance el pH final, especialmente si el proceso de enfriamiento de la carne es más lento (Lawrie, 1998). Existe un segundo sistema enzimático implicado en el incremento de la terneza dur

ante la maduración y es el de las catepsinas de los lisosomas. Estas enzimas proteolíticas (tipos B, D, H y L), presentan su pH óptimo por debajo de 6 (Etherington, 1984; Penny y Dransfield, 1979). Taylor et al. (1995) sugieren que las calpaínas podrían ser las responsables de la proteólisis en el músculo durante las primeras etapas, mientras que la contribución de las catepsinas sería principalmente a partir de los 6 días postmortem,

cuando el pH muscular es bajo. Existe otra fuente de actividad proteolítica en el músculo, llamada Proteosoma. Dicho complejo está presente en varios tejidos por lo que debe ser considerado al hacer referencia a la proteólisis de los mismos (Wilk y Orlowskyi, 1980).

La variabilidad en la tasa de maduración, depende del contenido de proteinasas dependientes de calcio, del contenido de catepsinas (y posiblemente de otros complejos enzimáticos tales como el proteosoma) y de sus respectivos inhibidores, así como de la relativa resistencia de las proteínas del músculo a la proteólisis y a la presión osmótica del mismo. Es muy importante la duración de esta etapa, siendo aconsejable por algunos investigadores, que sea como mínimo de 7 días en el caso del ganado vacuno. Se destaca que el incremento de la terneza es mínimo en músculos que han tenido acortamientos sustanciales durante el establecimiento del *rigor mortis* (Lawrie, 1998).

Factores que afectan la terneza

Según Koohmaraie (1996) el 40 % de la variabilidad de la terneza estaría explicada por factores ocurridos en el establecimiento ganadero, mientras que un 60-70 % de dicha variabilidad se explicaría por factores que ocurren durante el procesamiento.

Entre los diversos factores *pre mortem* que estarían afectando a la terneza de la carne, se destaca a la alimentación, la edad de los animales al momento de la faena, el manejo en los momentos previos a la misma, el temperamento y la raza de los animales, como los factores más relevantes desde el punto de vista del presente trabajo.

El estudio del efecto de diferentes estrategias de alimentación sobre las características de la canal y la calidad de la carne, ha arrojado resultados contradictorios durante muchos años. Algunos autores sostienen que la alimentación con elevados contenidos energéticos, proporciona carnes más tiernas (Asenjo, 1999). Las mayores ganancias diarias previas a la faena derivadas de un plano nutritivo superior y más energético, mejorarían la terneza por un aumento en la proporción de colágeno soluble. Cuando la tasa de crecimiento es elevada, el colágeno muscular de nueva síntesis tiene menor cantidad de uniones termolábiles (Sañudo y Campo, 1997) y también puede aumentar la actividad de los enzimas proteolíticos durante la fase postmortem (Blanchard et al., 1995; Miller et al., 1987; Sañudo y Campo, 1997; Thompson, 2002).

La alimentación energética provocaría incrementos en los niveles de glucógeno previo a la faena, permitiendo descender el pH a valores inferiores a 5,8 haciendo más tierna la carne y favoreciendo las demás características de la misma. Por otra parte y como ya se ha mencionado, los animales con mayores niveles de energía en la dieta, llegan al punto de faena más engrasados, lo cual implica un descenso más lento de la temperatura en la canal, una mejor protección ante el fenómeno cold shortening y una tasa de descenso de pH

adecuada. Wanderstock y Millar (1948) indican que la mayor terneza de los animales que se encuentran en altos planos de nutrición, estaría explicada por la mayor presencia de grasa intramuscular. Ésta diluiría el colágeno existente en el músculo en que se deposita.

Sin embargo, otros autores no han encontrado diferencias en la fuerza de corte de carne proveniente de animales con diferente ganancia de peso previo a la faena (Calkins et al., 1987; Cox et al., 2006, French et al., 2001; Moloney et al., 2000) y por el contrario, algunos autores han encontrado mejores valores de terneza en animales terminados en base a pasturas. Realini et al. (2004) registraron valores de 2.8 y 3.5 kg de fuerza de corte para carne de pasturas y concentrados, respectivamente. En esta misma línea, Oltjen et al. (1971) reportaron valores de terneza mayores en carne proveniente de animales terminados con pasturas, al compararla con la proveniente de animales en base a dietas de concentrado. Según Oltjen et al. (1971) las ventajas de los animales que presentan mayores ganancias de peso, es atribuida a la edad con que llegan a la faena. La concentración de colágeno no varía significativamente durante el crecimiento del animal hasta el sacrificio (Dikeman et al., 1986); sin embargo, el colágeno soluble disminuye con la edad (Cross et al., 1984; Sorensen, 1981), incrementando el número de enlaces covalentes termoestables que unen las moléculas individuales de colágeno, con lo que la dureza de base aumenta en relación a la carne de animales jóvenes (Tarrant, 1998). Dichas características del colágeno disminuyen la vulnerabilidad del mismo a ser atacado por enzimas (Goll et al., 1963). Contrariamente a lo que se supone que ocurre con el tejido conectivo, la dureza miofibrilar no aumenta con la edad del animal (Tarrant, 1988). Según Shorthose y Harris (1990) la edad tiene un efecto importante sobre la terneza, especialmente en animales con más de 30 meses de edad.

Algunos autores aseguran que la menor terneza de la carne de animales terminados a pasturas, se explica por la mayor duración del período de engorde, es decir, estaría también confundida con el efecto de la edad. Según Bidner et al. (1981) y Crouse et al. (1984) las diferencias en calidad de carne de animales provenientes de forrajes ó de concentrados, se minimizan cuando estos se comparan a un mismo punto de terminación tal como peso vivo, cobertura de grasa o grado de marmoreo.

Normalmente los períodos de terminación de animales en pasturas, son mayores que los que incluyen altos niveles de concentrado en la dieta. Es decir, las ganancias diarias son menores, el período de engorde es mayor y los animales alcanzan el punto de faena siendo más viejos. Todos estos factores, los cuales no son independientes, podrían explicar la mayor terneza de los animales de concentrado. Los resultados encontrados por Realini y Oltjen, ameritan mayores estudios, posiblemente implicando tanto factores *ante mortem* como factores *postmortem* que no han sido considerados hasta el momento.

El estrés sufrido por los animales en los momentos previos a la faena podría reducir el glucógeno del músculo *in vivo* (Tarrant, 1988; Warris, 1990). De esta manera, el pH no descendería lo suficiente, no lográndose la correcta acidificación del músculo como para lograr adecuados valores de terneza (Purchas et al., 1999; Watanabe et al., 1996). Por otra parte, la adrenalina que ocasiona el stress, inhibe el sistema proteolítico de las calpaínas que tenderiza el músculo postmortem (Sensky et al., 1998). Según Ouali et al. (2006) el estrés sufrido en forma previa al sacrificio, podría presentar un efecto negativo sobre la terneza, a través de la acción de ciertas proteínas que se encargan de prevenir la apoptosis o muerte celular. Dichas proteínas son producidas por las células del animal como forma de defensa, cuando éste se enfrenta a una situación de estrés. Por lo tanto, en el período inmediato a la muerte, podrían enlentecer el proceso de muerte celular constituyendo un obstáculo para la maduración. Según este autor, a las fases de rigor mortis y maduración, se debe agregar una fase más temprana, de iniciación de la muerte celular y deberían analizarse los cambios bioquímicos y estructurales que en ella ocurren (Figura 3).

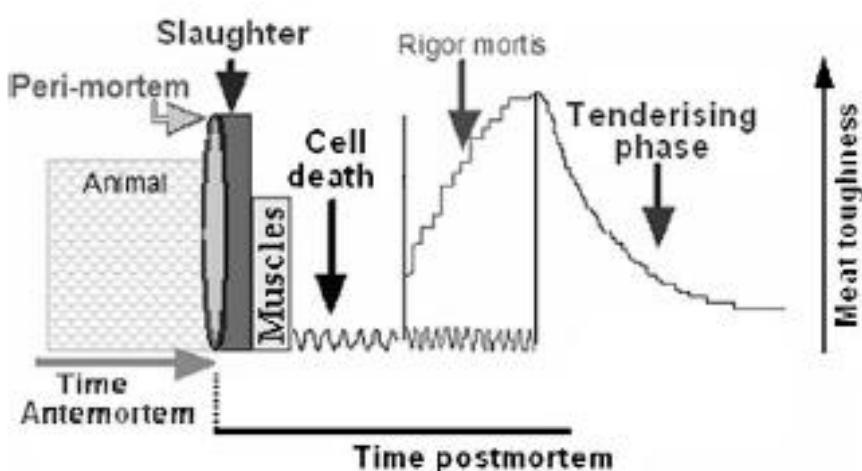


Figura 3. Fases en la conversión de músculo a carne.
Fuente: Ouali et al. (2006).

En este sentido, la respuesta individual de los animales antes las situaciones de estrés, podría presentar un efecto importante sobre las características organolépticas de la carne. Algunos autores han registrado incrementos de la terneza en animales de temperamento más calmo (Voisin et al., 1997), contrastando con los resultados de otros autores que alegan que la asociación fenotípica entre temperamento y terneza es débil o inexistente (Burrow et al., 1999; Kadel et al., 2006; King et al., 2006; Petherick et al., 2002). Dichos resultados contradictorios pueden deberse a diferentes factores, destacándose entre ellos la posible utilización de métodos subjetivos, no estandarizados y posiblemente inadecuados para la determinación del temperamento (ver apartado 2.2.7 y 2.2.7.3.). Tampoco han sido

concluyentes los estudios que relacionan el temperamento con la incidencia de cortes oscuros o pH último (Fordyce et al., 1988 b; Petherick et al., 2002).

La carne de las razas índicas y continentales es menos tierna (mayor fuerza de corte y menor clasificación sensorial) y más variable en terneza que la carne de razas de origen británico (Wulf et al., 1997), independientemente del ambiente en el cual el animal produce (Depetris, 2000). Algunos autores atribuyen estas diferencias a un posible mayor engrasamiento de las razas británicas (Peluffo y Monteiro, 2002). Según Franco et al. (2002) la fuerza de corte tiende a aumentar a medida que aumenta la proporción de sangre índica. Esto sería el resultado de una menor proteólisis, debido a que el mayor nivel de las calpastatinas determina una menor velocidad y profundidad de los efectos de maduración (Santini et al. 2003, Teira, G. 2004). La raza también tiene un efecto significativo en el contenido total e insoluble de colágeno, que puede ser más importante que el peso o el sistema de producción (Shackelford, et al., 1991).

2.1.2.3. Factores de calidad social

2.1.2.3.1. Bienestar animal

El bienestar de los animales atribuye valor a la carne en forma directa (calidad social), destacándose además el efecto negativo que puede ocasionar el estrés sobre los factores sensoriales, bioquímicos e higiénicos. Este se debe a la ocurrencia de procesos anormales en la transformación de músculo en carne, donde podría afectarse el pH, el color, la jugosidad y la terneza, entre otros (del Campo, 2006).

Dado que el Bienestar animal constituye el segundo pilar fundamental de este trabajo, se profundizará en su definición así como en las formas de su determinación, en el apartado 2.2.

2.2. Bienestar animal

La temática del Bienestar animal (BA) está muy ligada a la existencia y evolución de las diferentes posturas éticas a lo largo del tiempo. Estas han defendido una u otra de las concepciones del BA emanadas de su filosofía y han provocado grandes debates o controversias en lo que tiene que ver con el reconocimiento social o estatus moral otorgado a los animales, en función de su capacidad o no de experimentar emociones (del Campo, 2006). Hoy, el pensamiento occidental ha logrado un consenso generalizado en la determinación de criterios de base relacionados al BA tales como: "evitar el sufrimiento innecesario" y "si algo le hace daño al ser humano, es probable que también le haga daño al animal". Si bien como ciencia o disciplina el BA ha sido desarrollado recientemente, la

preocupación por el buen trato a los animales data de épocas muy antiguas. Respecto a normativas, se destaca que la preocupación internacional por el BA se ha visto plasmada en legislaciones desde 1876 en Gran Bretaña y a partir de 1960 en Estados Unidos.

A partir de 1970 comienzan los primeros estudios científicos sobre el tema. La comunidad científica internacional consideraba que el BA estaba íntimamente ligado a la presencia de ciertos procesos fisiológicos, especialmente aquellos relacionados al estrés. Sin embargo, ya en 1964, tanto en producción intensiva como en investigación, había comenzado a hablarse de “sufrimiento” (Harrison, 1964). En años sucesivos, el Bienestar animal pasa a ser un concepto más amplio que incluye tanto el buen estado físico de los animales, así como el estado mental, surgiendo diferentes concepciones del término, lo que ha dificultado la formulación de una definición científica precisa y única. A partir de la década del 80, se logran importantes avances en la investigación sobre esta nueva disciplina, surgiendo grandes discrepancias en la comunidad científica, acerca de los mejores indicadores a la hora de evaluar el Bienestar animal.

2.2.1. Concepto de Bienestar animal

El BA no es un concepto puramente científico sino que surge desde la sociedad para expresar una preocupación ética acerca del tratamiento de los animales (Duncan y Fraser, 2005). El BA es un estado relativo a los intentos del animal de adaptarse al medio que lo rodea, el cual incluye todo lo que tenga que hacer para abordarlo, el grado en que lo logra o falla, su salud y los sentimientos asociados a ello (Broom, 1986). Es una característica inherente al individuo en un determinado momento, que puede ser establecida objetivamente y que variará desde muy bueno a muy pobre. Cuando las condiciones son difíciles, los animales utilizan diversos métodos tratando de contrarrestar los efectos adversos o adaptarse a ellos, pudiendo encontrarse en tres diferentes situaciones según su grado de adaptación al ambiente (Velarde y Manteca, 2000):

- 1) el ambiente es inadecuado, la adaptación no es posible por lo que el animal morirá o sufrirá lesiones causadas por el ambiente
- 2) el animal consigue adaptarse al ambiente pero esa adaptación supone un costo para el mismo. Este costo puede ser el resultado de una respuesta fisiológica de estrés particularmente intensa o de cambios de comportamiento inducidos por el ambiente
- 3) el animal se adapta al ambiente sin que le suponga un costo, en ese caso el bienestar es adecuado

2.2.2. Escuelas de pensamiento

Aún en la actualidad, es posible distinguir 3 escuelas de pensamiento respecto al Bienestar animal y sus formas de determinación (Fraser et al., 1997).

Escuela de los Sentimientos

Esta corriente de pensamiento sostiene que el Bienestar animal tiene que ver con la ausencia de experiencias emocionales negativas (sufrimiento) y probablemente con la presencia de estados emocionales positivos (placer) (Duncan y Fraser, 2005). Considera que todos los organismos vivos presentan necesidades de supervivencia, crecimiento y reproducción, y que los organismos superiores (vertebrados e invertebrados superiores) además, experimentan sentimientos. Estos estados afectivos motivan el comportamiento y van más allá del comportamiento típico de estímulo – respuesta, en el que no hay conciencia o procesos mentales involucrados. De acuerdo a este enfoque, el rol de la ciencia debería centrarse en el estudio y la comprensión de dichas experiencias subjetivas.

Escuela Biológica funcional

El Bienestar animal se define en términos del funcionamiento normal y satisfactorio de los sistemas biológicos (homeostasis). Este enfoque sostiene que el bienestar de los animales está determinado por la ausencia de respuestas de estrés (al menos en el largo plazo), por la capacidad de adaptarse al ambiente que los rodea y por la satisfacción de las necesidades biológicas. Es así que se vería afectado por la incidencia de enfermedades, lesiones o mal nutrición, y sería adecuado con buenos niveles de crecimiento y reproducción, con un normal funcionamiento de los procesos fisiológicos y comportamentales, y con una longevidad adecuada. Esta escuela de pensamiento está muy influenciada por el concepto de estrés (ver apartado 2.2.3.) desarrollado por Selye (1950). Cualquier incremento o alteración de los indicadores de estrés, implicaría una reducción del BA (Broom y Johnson, 1993).

Escuela de los Comportamientos naturales

Esta corriente de pensamiento sostiene que el BA se logra si los animales se desarrollan en ambientes “naturales” en los que se les permite desarrollar sus comportamientos “naturales”. Dicho enfoque no ha logrado ponerse de acuerdo con los anteriores, siendo criticado por diversos autores, quienes argumentan y han demostrado que es una perspectiva parcial e insuficiente, alegando que los comportamientos naturales no siempre son indicadores de BA. Por el contrario, la vida en condiciones naturales puede implicar en muchos casos

problemas severos y fatales, enfrentando a los animales a una permanente lucha por la sobrevivencia (Dawkins, 1980; Poole, 1996).

Siempre que los comportamientos naturales y las experiencias subjetivas promuevan el correcto funcionamiento de los sistemas biológicos, las diferentes escuelas de pensamiento coincidirán en sus acepciones y conclusiones sobre el BA. Duncan y Fraser (2005) sostienen que de hecho, la investigación ha demostrado que la escuela de los sentimientos y la escuela biológica funcional, se corresponden. Muchos autores argumentan que si bien las experiencias subjetivas son las que realmente definen la calidad de vida de un animal, estas son eventos privados, difíciles de establecer en forma científica. En este sentido, Duncan y Pethericks (1991) sostienen que en la actualidad no se conoce virtualmente nada acerca de los procesos mentales subjetivos de los animales. Es por ello que diversos autores han adoptado la corriente biológica funcional, ya que esta permite el logro de información adecuada y relevante relativo a la calidad de vida de un animal (Gonyou, 1993). Con este mismo criterio, en el presente trabajo de tesis se ha adoptado dicha corriente de pensamiento.

La lectura más relevante de los avances logrados por la ciencia, es que el Bienestar animal ha dejado de ser un aspecto sentimental o subjetivo para pasar a ser un aspecto objetivo y cuantificable que combina diferentes dimensiones del animal y/o del ambiente, y que su caracterización o mejora debe realizarse en base a indicadores acordes al contexto en el cual se trabaja. El avance del conocimiento científico permitiría resolver las diferencias o desacuerdos entre los diversos enfoques del BA. Sin embargo, es importante destacar que la ciencia no podrá alcanzar una concepción totalmente objetiva al momento de integrar las diferentes medidas, ya que misma necesita tanto del conocimiento, como de sentido común y de la emisión de juicios de valor (Webster, 2005). Una vez que se ha generado la evidencia objetiva relativa al BA, se deberán tomar decisiones de tipo ético para su integración (Broom y Jhonson, 1993; Dawkins, 1980; Duncan y Dawkins, 1983; Duncan y Fraser, 2005).

2.2.3. Estrés

Cualquiera sea la definición que se tome del Bienestar animal, se considera que el estrés independientemente de su factor generador, conlleva a situaciones de inadecuado bienestar. El estrés es la respuesta del organismo ante agentes nocivos o estresantes (Selye, 1950). Se considera estrés a cualquier amenaza a la homeostasis, ya sea que dicha amenaza sea real o simplemente percibida por el animal (Rivier y Riviest, 1991). Entre los

agentes estresantes más comunes se incluyen las variaciones en el medio externo (frío, calor, falta de alimento, falta de agua, agresión, inmovilización), determinadas alteraciones psicológicas (miedo, ansiedad, aburrimiento, frustración, soledad, depresión) así como también algunos cambios en el medio interno: enfermedades, lesión tisular, dolor. En la mayoría de los casos se dan combinaciones de los mismos. En general, la situación de estrés activa el sistema simpático adrenal, el eje adreno corticotrópico (HPA), el sistema inmunitario, y altera el comportamiento (Moberg, 2000) (Figura 4).

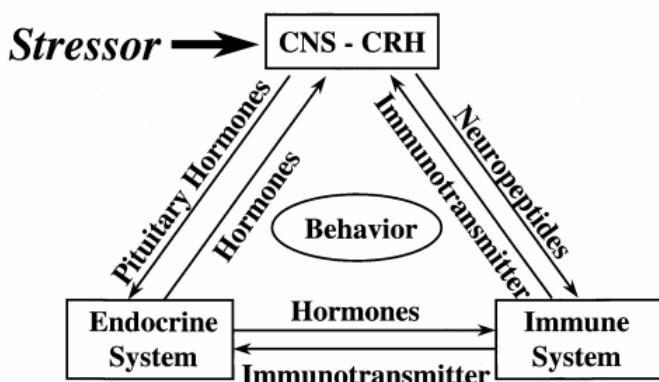


Figura 4. Comunicación entre el sistema nervioso central, el sistema endócrino y el sistema inmunitario (integración neurobiológica del estrés). CRH: factor removedor de hormona corticotrópica. ACTH: hormona corticotrópica. Fuente: von Borell, 2001.

En respuesta al factor estresante, se dispara una respuesta en el animal que involucra al sistema nervioso simpático y al eje HPA, lo cual resulta en una cascada de eventos hormonales. Selye (1946) distingue 3 fases en dicha respuesta ante situaciones de estrés:

2.2.3.1. Síndrome de alarma, lucha o huída

En primer lugar, se liberan a la sangre grandes cantidades de adrenalina y noradrenalina desde la médula adrenal (descarga simpática). Esta descarga da lugar a la llamada reacción simpática de alarma o síndrome de lucha o huída, la cual sucede en segundos. Cannon (1914) la describe como la reacción o síndrome de emergencia, en la que se da una respuesta corta que involucra los factores hormonales mencionados (catecolaminas). Estos promueven la remoción y destrucción de glucosa que proviene del hígado para hacerla disponible en el músculo. La glucosa que es la fuente principal de energía del organismo y se pone en movimientos desde sus zonas de almacenamiento (hígado). La sangre que transporta a la glucosa y al oxígeno, se desvía de los órganos no esenciales para la actividad motora y se dirige rápidamente hacia los órganos necesarios tales como el corazón, músculos esqueléticos y cerebro. Se priorizan actividades fisiológicas necesarias para el beneficio inmediato, postergando otras como digestión, crecimiento, reproducción,

etc. (Sapolsky, 1990). Como consecuencia entonces a la descarga simpática, se produce un aumento de la presión arterial, del flujo sanguíneo a los músculos motores, del metabolismo celular, de la concentración de glucosa en sangre, de la fuerza muscular y de la actividad mental, disminuye la percepción del dolor, y ocurre broncodilatación y dilatación pupilar (García Sacristán, 1995).

2.2.3.2. Resistencia o fase de adaptación

En esta fase (Síndrome general de adaptación) el hipotálamo segregá corticotrofina (CRH) y argenina vasopresina (AVP), las cuales estimulan a la adenohipófisis a segregar la hormona adrenocorticotrófica (ACTH). Esta última estimula la secreción de glucocorticoides por parte de la corteza adrenal. Los glucocorticoides presentan diversas funciones en el organismo (permisivas, estimulatorias, supresivas, otras) destinadas al mantenimiento de la homeostasis (Sapolsky et al., 2000). Es así que durante la fase de recuperación o adaptativa de la respuesta al stress, los corticosteroides promueven la resíntesis de glucógeno del hígado a partir de glucosa circulante, manteniendo los niveles de glucogénesis (Warris, 2000). El cambio en la prioridad de actividades fisiológicas necesarias para enfrentar la situación estresante, es lo que se denomina costo biológico del estrés (Moberg, 2000). En la mayoría de las situaciones, dicho costo es irrelevante, ya que son respuestas de poca duración que son en principio útiles para el animal, ya que le permiten enfrentarse a la nueva situación. Cuando la situación de estrés es excesivamente intensa o prolongada, dicho costo biológico puede significar una amenaza significante para el organismo (Matteri, Carroll, Dyer, 2000). Mason (1971) sostiene que la activación del eje adrenocorticotrópico dependerá del grado de involucramiento emocional del animal. Es decir, para que ocurra la activación de dicho eje, es necesario que el animal perciba la situación como estresante.

2.2.3.3. Agotamiento: estado prepatológico y eventual muerte

Si el desafío es demasiado severo, complejo o duradero y el animal no puede desarrollar la respuesta apropiada, puede no lograrse esa adaptación, dando lugar a la presente fase. Los agentes estresantes a través de su impacto en los sistemas neuroendocrino, autonómico y sistema nervioso central, pueden influir en la función inmunológica. Eso dependerá de las diferentes situaciones y de la vulnerabilidad de cada individuo (Anisman, 2002).

La metabolización continua de glucosa sin oportunidad de almacenarla nuevamente, hace que los tejidos se atrofien y se fatiguen. Los cambios cardiovasculares inducen la aparición de hipertensión que puede lesionar el corazón, vasos sanguíneos y riñones. Con el tiempo, se ven afectado el crecimiento y la reaparición de tejidos, se reduce la fertilidad y se produce una depresión del sistema inmunitario (Selye, 1936). Cualquiera sea la ruta que sigan los mensajes inmunológicos para alcanzar y ser interpretados por el sistema nerviosos central, el

hecho de que afecte los procesos del SNC, determinaría que los procesos inmunes alterarían también el comportamiento (Banks, 2001).

2.2.4. Formas de determinación y medición del Bienestar animal

El estudio de las potenciales fuentes de estrés y su impacto en el bienestar de los animales, requiere de un enfoque multidisciplinario e integral, en el que se deberán considerar y combinar diversos tipos de indicadores respecto al funcionamiento de los sistemas corporales, el sistema inmunológico, variables productivas, las respuestas fisiológicas ante el stress y variables comportamentales individuales que reflejen entre otras cosas, los sentimientos de los animales (Terlow et al., 2005). Entre los diversos indicadores de BA en animales en producción, se destaca dentro de los respectivos ítems o disciplinas, a aquellos más relevantes para el presente trabajo de tesis.

2.2.4.1. Productividad

La productividad es una medida indirecta del BA que evalúa el efecto de los agentes adversos (costo biológico) o favorables sobre la capacidad de crecimiento y ganancia de peso o engorde (evolución del área de ojo del bife, peso vivo, condición corporal), reproducción (indicadores reproductivos) y sanidad (incidencia de enfermedades, presencia de lesiones, mortalidad). La estimación del BA desde una perspectiva productiva es práctica, ya que no implica la obtención de medidas adicionales a las que regularmente se registran en una empresa agropecuaria (Bennet et al., 2000). La productividad es muy relevante para el establecimiento del status de calidad de vida de una animal ya que certifica que al menos ciertos aspectos o necesidades estarían satisfechos. Sin embargo, debe considerarse que es un concepto económico y como tal, conceptualmente puede y suele ser positivo, en situaciones de inadecuado bienestar. Muchos individuos podrían sufrir e incluso morir y el proceso como un todo continuaría siendo rentable (Rollin, 2003). El efecto negativo del estrés ocurre a nivel del individuo, mientras que la productividad y la rentabilidad se registran a nivel de la unidad de producción (Appleby and Hughes, 2005; Mench, 1992).

2.2.4.2. Salud y mortalidad

La salud física es indudablemente una condición necesaria para el BA. Sin embargo, el concepto de salud no se refiere a la mera ausencia de enfermedad, sino al completo estado de bienestar de un individuo, incluyendo su salud mental y física. El entendimiento de las relaciones entre salud y BA dependerá de las inferencias que se realicen acerca de las experiencias subjetivas tales como el dolor y la ausencia de confort (Appleby y Hughes, 2005). El status sanitario entonces constituye un buen indicador de BA, debiendo constituir uno de los objetivos fundamentales de cualquier sistema de producción (Gottardo, 2004).

La mortalidad es un claro indicador de falta de BA, no solamente porque los animales que han muerto obviamente no han logrado adaptarse al ambiente, sino porque grandes pérdidas productivas asociadas al ambiente, muestran que incluso aquellos animales que sobreviven, podrían estar teniendo serias dificultades para lograrlo (Manteca and Velarde, 2007) ya que están sometidos a las mismas condiciones deficientes (Warris, 2003).

2.2.4.3. Indicadores fisiológicos

Algunos signos de inadecuado bienestar pueden obtenerse a través de la evaluación de variables fisiológicas relacionadas al estrés. Entre ellas se encuentra la respuesta mencionada del sistema nervioso simpático (SNS) tales como el incremento del ritmo cardíaco, la frecuencia respiratoria, la temperatura corporal e incremento de la actividad adrenal (catecolaminas). Los cambios fisiológicos resultado de la activación del SNS, serían más específicos o certeros en la determinación de estados inmediatos o tempranos de la respuesta al estrés (Mellor et al., 2000). La concentración de catecolaminas en plasma no se utiliza normalmente debido a que presentan una vida útil muy corta y estas son metabolizadas rápidamente, una vez que se encuentran en el torrente sanguíneo (Webster, 2005). A los efectos del presente trabajo de tesis, se destacan a continuación algunos indicadores asociados a la respuesta de estrés del organismo, tales como el nivel de glucocorticoides en sangre y heces, proteínas de fase aguda en sangre, indicadores de daño muscular, de estado nutricional y del estado de hidratación de los animales.

Glucocorticoides

Una de las variables fisiológicas más utilizadas para estudiar la respuesta al stress es la concentración de glucocorticosteroides en plasma o suero, evaluando de esta manera la actividad adrenocortical. La concentración de glucocorticosteroides en plasma o suero presenta ciertos inconvenientes metodológicos, entre los que se citan:

- 1- los propios procedimientos implicados en la extracción de la muestra de sangre pueden constituir un factor más estresante aún que el que se intenta cuantificar (Hopster et al., 1999). El cortisol es tiempo dependiente llegando a los valores pico a los 10-20 minutos luego de iniciado un acontecimiento estresante (Lay et al., 1992). Es así que los procedimientos deben realizarse rápidamente (Grandin, 1997)
- 2- se necesita un muestreo frecuente debido a las fluctuaciones de dichos valores durante el día (variación circadiana), e incluso entre minutos (Mulleeder et al., 2003)

- 3- su interpretación debe manejarse en el contexto en que se generó, debido a que diversos estímulos ambientales que no comprometerían el BA, pueden producir un incremento de su concentración en sangre (cópula, alimentación, etc.)

El estrés crónico en general no es medible a través de cambio en el eje HPA o del sistema simpato adreno medular (SNA), debido a eficientes mecanismos de retroalimentación de ambos sistemas. Para cuantificar el stress crónico puede utilizarse la medición de metabolitos de cortisol fecal, con lo cual se corrige o solucionan los problemas metodológicos del cortisol en plasma o suero, mencionados anteriormente (fluctuaciones normales, animal inmovilizado con el probable consecuente incremento de actividad del eje, incremento debido a otro tipo de actividades) (Palme y Mostl, 1997; Palme et al., 1999; Palme et al., 2000). Los metabolitos fecales en rumiantes son excretados en forma muy posterior a su producción (Palme et al., 1999). El tiempo requerido para la excreción de los metabolitos, es cercano al necesario para el tránsito regular de la ingesta, pasando por el conducto biliar y el recto (Morrow et al., 2002). La concentración de cortisol en heces de rumiantes, estaría determinando los niveles sanguíneos de aproximadamente 12 horas previo a la extracción de las muestras (Mostl et al., 2002). Estos estarán reflejando niveles promedio de cortisol circulante durante un período de tiempo y no en un momento específico (muestra puntual), por lo que ofrecerán una medida más acertada de los niveles de cortisol en el largo plazo (Harper y Austed, 2000).

Proteínas de fase aguda

Las proteínas de fase aguda (PFA) son un grupo de proteínas que cambian su concentración en sangre de animales que son sometidos a desafíos internos o externos tales como infecciones, inflamaciones, traumas derivados de cirugías o situaciones de estrés. Estas proteínas se consideran componentes innatos no específicos del sistema inmune, que se encuentran implicados en la recuperación de la homeostasis y en el impedimento del crecimiento microbiano, previo al desarrollo de inmunidad adquirida ante un determinado desafío (Murata et al., 2004). La concentración circulante dependerá de la severidad del desorden así como del daño tisular ocasionado. Su cuantificación podría proveer de valiosa información para la realización de diagnósticos tempranos de enfermedades (Murata et al., 2004), pero también como herramientas de vigilancia o control del estado sanitario y del Bienestar animal en condiciones de producción (Eckersall, 2000). En respuesta a un factor estresante, éstas incrementan su concentración en sangre (Conner et al., 1988). Son producidas por los hepatocitos, que son estimulados directamente por citokininas proinflamatorias tales como IL-1, IL-6 y el factor de necrosis tumoral (tumor

necrosis factor) (Richards et al., 1991; Breazile, 1996). Estas citokininas proinflamatorias son altamente pleiotrópicas, existiendo evidencia significativa de que estarían implicadas directamente en la inhibición del crecimiento (Johnson, 1997).

Arthington et al. (2005) compararon respuestas de estrés en terneros destetados en forma tradicional y en forma temprana, registrando incrementos de PFA e inhibición del crecimiento, sin incidencia de enfermedades. El mecanismo de inducción de las PFA en respuesta al estrés aún no es claro, pero sí que la activación del eje HPA ante señales de estrés, puede ser un disparador de la producción sistémica o local de citoquinina (cytokine), aumentando así la síntesis hepática de PFA y su vuelco al torrente sanguíneo (Murata et al., 2004).

Indicadores de fatiga o daño muscular

La Creatin fosfoquinasa (CPK) es una enzima específica del músculo y es liberada al torrente sanguíneo cuando existe daño muscular, como puede ser el derivado de un ejercicio violento que puede ocurrir durante el transporte o la espera en matadero. En el animal vivo, su incremento en el torrente sanguíneo puede ser un indicador muy útil de daño de la membrana muscular, de excesiva actividad muscular (Warris, 2000) u otro tipo de daño (Lefebvre et al., 1996). Si bien no es una medida directa de estrés, es una consecuencia que indirectamente puede asociarse a situaciones de estrés o pérdida de bienestar. Berg y Haralambie (1978) sostienen que el incremento de CPK en sangre implica el movimiento constante tanto de músculos voluntarios así como de aquellos controlados por el sistema nervioso autónomo (corazón, pulmones).

Indicadores de estado nutricional

En bovinos, la concentración de ciertos metabolitos en sangre tales como los ácidos grasos libres (AGL) y los cuerpos cetónicos o hidroxibutirato, puede verse afectada por períodos de restricción alimenticia, constituyendo de esta manera indicadores de la movilización de reservas corporales como consecuencia de un déficit energético (Baird et al., 1972; Bergman, 1971; Shaw and Tume, 1992; Ward et al., 1992) o de otras situaciones estresantes.

Indicadores de estado de hidratación

Los valores de hematocrito pueden verse afectados por la falta de alimento y/o agua prolongados. El incremento de los valores de hematocrito en respuesta a un factor estresante agudo, es una consecuencia de la contracción esplénica provocada por la liberación de catecolaminas.

2.2.4.4. Etología - comportamiento

La relevancia del comportamiento como herramienta para la determinación del bienestar de los animales ha sido explícita por el Comité Brambell desde 1965 (Brambell Committee, 1965). El comportamiento es la herramienta que los animales utilizan para cambiar y controlar su entorno, por lo que es muy útil en la obtención de información acerca de sus necesidades, preferencias y estados internos (Mench y Mason, 2005). Es posible el estudio de cambios de comportamiento, presencia de comportamientos anormales, o la realización de test de preferencia y motivación. Para cualquiera de estas técnicas, es necesario conocer el repertorio comportamental característico de la especie en estudio (patrones de alimentación, bebida, descanso, socialización, autocuidado), el contexto en que este ocurre, así como el conocimiento y la distinción de los comportamientos típicos individuales de los grupales (Mench y Mason, 2005).

Los bovinos en general no suelen presentar comportamientos anormales tales como estereotipias, pero se deberían evaluar posibles indicadores de aburrimiento y/o frustración especialmente en los sistemas más intensivos de producción.

2.2.5. El Bienestar animal en los sistemas de producción ganaderos

Diversos factores influyen en el bienestar de los animales a nivel de producción. Algunos de ellos repercutirán sobre la vida cotidiana del animal, afectando su comodidad y bienestar en el corto y mediano plazo (situación climática, exposición a depredadores, prácticas rutinarias, mezclas de grupos, etc.). Sin embargo, la mayoría de las decisiones tomadas en el establecimiento estarán afectando el bienestar de los animales también en el largo plazo así como la calidad de los productos obtenidos, ya que serán determinantes entre otras cosas, del temperamento de los animales y por tanto de sus respuestas al manejo. Entre dichas decisiones se destaca la genética, el sistema de alimentación, el manejo sanitario y la aplicación de buenas prácticas de manejo. En general, un aumento en el grado de bienestar implica un aumento de la productividad, pero dicha relación no es lineal (Velarde y Manteca, 2000). Cuando la situación inicial o punto de partida es muy mejorable, incrementos o mejoras relativamente pequeñas en el bienestar, suponen aumentos considerables en la productividad. Sin embargo, a medida que la situación mejora, aumentos similares en el bienestar, suponen menores aumentos en la productividad (Velarde y Manteca, 2000). Por otra parte, mejoras en el BA suponen un incremento de los costos de producción. La magnitud de ese incremento dependerá de la magnitud del cambio que se desea realizar, así como como de la situación particular o punto de partida de cada establecimiento. En general, cuanto mejor sea la situación de partida, más caras resultarán las mejoras respecto al BA (Velarde y Manteca, 2000).

2.2.5.1. Particularidades del Bienestar animal en condiciones extensivas de producción

En general existe la idea errónea de que los sistemas extensivos de producción a cielo abierto no presentan inconvenientes desde el punto de vista del Bienestar animal. Si bien dichas condiciones de producción los posicionan favorablemente en varios aspectos, especialmente en lo que tiene que ver con las expresión de los comportamientos naturales en los animales, existen algunas amenazas reales asociadas a las características de los sistemas extensivos.

Entre los diversos factores que estarían amenazando el Bienestar animal en condiciones extensivas, se encuentra la posible subalimentación y/o subnutrición debida a la estacionalidad de la producción de forraje, la inadecuada relación entre la carga animal y el forraje disponible y/o a la deficiencia de ciertos minerales esenciales y elementos traza en las pasturas (McCosker y Winks, 1994). Esto hace que en ciertas épocas del año puedan ocurrir pérdidas de peso y condición corporal.

La supervisión que existe por parte del hombre no es tan frecuente como en la producción intensiva, por lo que existe un mayor riesgo de que los animales padeczan enfermedades, lesiones, muerte. Por otra parte, en aquellas situaciones en que los animales tienen contactos esporádicos con el hombre, es más difícil el logro de evoluciones favorables del temperamento, principalmente en animales agresivos, destacándose el efecto que ello podría presentar sobre la productividad (Fordyce et al., 1988 a; Voisinet et al., 1997; Patherick et al., 2002) y la calidad de la carne.

La exposición a situaciones climáticas adversas, la frecuente carencia de abrigo y sombra, algunas características de prácticas de manejo tradicionales (marcación, descorne, castración, señalada y esquila en ovinos, otros), la alta mortandad neonatal en ovinos, así como el manejo en general (especialmente el tradicional uso del perro), irían en detrimento del bienestar de los animales, en la medida en que no se optimicen.

Otro factor diferencial en los sistemas extensivos es la presencia de predadores en ciertas regiones, lo cual se ve agravado por la carencia de vigilancia constante ya sea debido al sistema de manejo, a factores topográficos, lejanía, costos, otros (del Campo, 2006).

2.2.5.2. Manejo

Una característica común de todas las especies animales que han sido domesticados para la producción ganadera, es que son animales sociales habituados a vivir en grupos estables, con una determinada jerarquía social dentro de esos grupos. La estabilidad de dicha jerarquía se refuerza a través de señales anatómicas, fisiológicas y comportamentales que

indican a cada individuo el lugar que ocupa dentro del grupo (Boivin et al., 2003). Quizás no sería tan relevante saber si el animal ve al hombre como parte de su grupo, situándolo en una postura dominante, sino que independiente a ello, se logre una verdadera comunicación entre ambos, que el lenguaje sea apropiado al ambiente y diseñado de forma de minimizar el estrés. El vocabulario de señas necesario para lograr una comunicación con los animales de granja, sin duda es menor que lo que se ha logrado en las relaciones hombre-perro u hombre-caballo, ya que los contactos necesariamente son menores. Sin embargo, los principios serán idénticos: las señales deben ser informativas, no deberán ser ambiguas y en un lenguaje que el animal pueda entender (Webster, 2005).

Existe abundante información sobre el efecto de la calidad del manejo en el BA y la productividad (Boivin et al., 1994; Breuer, 2003; Grandin, 1997; Hemsworth, 2003; Hemsworth y Coleman, 1998; Lensink, et al., 2000). Todos estos autores coinciden en que animales que reciben un manejo adecuado, son menos susceptibles al estrés generado por las diversas rutinas de una empresa ganadera que involucran la presencia del hombre. Dicho efecto se ve reflejado no solo en el comportamiento sino en la productividad de los animales (Breuer et al., 2000; Rushen et al., 1999). El miedo es un factor que puede provocar descenso en el bienestar de los animales y en la productividad a nivel comercial. Se supone que la depresión del crecimiento es consecuencia de una serie de respuestas frente al stress agudo y crónico debida a la presencia del hombre. (Barnett, et al., 1983; Hemsworth et al., 1998).

El diseño de las facilidades/instalaciones, deberá basarse en el comportamiento de los animales, permitiéndoles el libre movimiento y circulación dentro de las mismas (Grandin, 1993). De esta manera se facilita el manejo (Boissy y Bouissou, 1988; Boivin et al., 1994) y se disminuyen los riesgos de sufrir accidentes, así como de destruir instalaciones.

2.2.6. El Bienestar animal en las etapas previas a la faena

En las horas previas y durante la faena, se debe minimizar el stress ocasionado a los animales, teniendo también en cuenta que pueden afectarse en forma negativa las características organolépticas y tecnológicas de la carne (Monin, 1998). En las etapas previas al sacrificio, se valoriza y capitaliza el efecto que pueden tener las decisiones empresariales y la calidad del manejo aplicado a los animales a lo largo de toda su vida, así como durante el transporte y la faena específicamente. Existen numerosos trabajos que han relacionado el trato que reciben los animales en las fases previas al sacrificio (establecimiento, transporte, ferias ganaderas, y espera en frigorífico) con la calidad de canal y carne que se obtiene (revisión: Ferguson et al., 2001). Los mismos aseguran que

mejoras en el manejo a nivel de toda la cadena de producción, asociadas a un mejor Bienestar animal, se traducen en una mayor calidad de la canal y la carne.

Son numerosos los factores que pueden promover el metabolismo del músculo durante esta etapa. En primer lugar, la tensión y la excitación provocados por el viaje, seguido de la actividad que generalmente ocurre en los corrales de espera, la privación de alimento y/o agua, el momento de traslado desde los corrales hasta el cajón de noqueo y finalmente el propio proceso de insensibilización, que causa tensiones musculares durante la contracción en el noqueo (fase tónica) y las convulsiones que le siguen (fase clónica) (Gregory, 2006).

2.2.6.1. Transporte

Durante el transporte, los animales se enfrentan a diversos factores novedosos y estresantes en un período de tiempo relativamente corto (posible mezcla de animales, carga y descarga, movimiento del vehículo, falta de agua y alimento, cambios de temperatura y humedad, etc.) (Jacobsen et al., 1993; Schaefer et al., 1997). Según Tarrant (1988) el stress del transporte puede ser leve o moderado, no poniendo en peligro el bienestar del animal, o puede ocasionar respuestas extremas que terminan en un estado de angustia. Algunos autores sostienen que el transporte es uno de los factores más estresantes para el ganado bovino (Marahrens et al., 2003). Por el contrario, Honkavaara (2003) afirma que cuando se utilizan vehículos nuevos y especialmente equipados, el ganado puede ser transportado por 8 a 14 horas sin que el transporte tenga efecto alguno sobre el Bienestar animal, los niveles de stress y la calidad de la canal y la carne. En este mismo sentido, Ishiwata et al. (2008) no encontraron diferencias en las concentraciones de cortisol en sangre, pre y post transporte, afirmando por tanto que éste no implica una severa situación de estrés en bovinos. Fazio et al. (2005) sugieren que las variaciones de cortisol en sangre luego de viajes de corta distancia, dependerían probablemente del contacto que los animales hayan tenido previamente con los operarios.

Estudios realizados por Lensink et al (2000) muestran que el manejo positivo o adecuado de los terneros durante su crianza, reducen la respuesta emocional frente al manejo y el transporte, por lo que ocurren menos incidentes negativos. Además de mejoras en las respuestas a la presencia del hombre, estos animales necesitan menos esfuerzo para ser cargados y descargados en el camión, presentan menor ritmo cardíaco en el momento de la carga y sufren menos incidentes en la planta de faena, comparados con los animales que son manejados en forma negativa. Asimismo, muestran menores valores de pH y mejores valores de color en carne. La reducción en la respuesta emocional sería la explicación de las mejoras en la calidad de carne de esos animales (Lensink et al., 2000).

Durante el transporte, es normal que ocurra una pérdida de peso en los animales debido al ayuno prolongado y al vaciado del contenido intestinal. Dichas pérdidas o mermas están generalmente estimadas para cada especie, dependiendo del sistema de alimentación, ayuno previo al embarque, distancia al matadero, tiempo y condiciones del transporte, tiempo y condiciones de la espera, sexo, categoría, etc.. Es así que los animales deben ser preparados para el transporte en lo que tiene que ver con el balance energético y de fluidos corporales (Marahrens et al., 2003). Cuando la duración del transporte es muy larga, se deben prever los intervalos de alimentación para mantener las diversas necesidades fisiológicas y el comportamiento de los animales durante el mismo.

Los procesos de carga y descarga provocan un stress adicional al del viaje. En un estudio realizado por María et al. (2004) se observaron 40 viajes a lo largo de España durante un año, de manera de diseñar un método objetivo para determinar el stress. En más de la mitad de los casos, tanto de embarque como de desembarque, hubo resistencia por parte de los animales, resbalones y vocalizaciones. Los enfrentamientos y las montas fueron de poca importancia y las vocalizaciones y caídas fueron más frecuentes en el embarque que en el desembarque. Los resultados de concentración de cortisol en plasma, glucosa y lactato, así como la actividad de la creatinquinasa y el pH de la carne a las 24 horas post mortem, confirmaron que el embarque es más estresante que el desembarque. En este sentido, resultados obtenidos por otros autores en Gran Bretaña, indican que el embarque y el desembarque son las etapas más estresantes del viaje para ovinos y bovinos (Knowles, 1999; Trunkfield y Broom, 1990). Por otra parte, Kenny y Tarrant (1987) reportaron que el viaje en sí fue más estresante que la carga y la descarga. Estos resultados contradictorios se explicarían por el mayor contacto con humanos que habían tenido los animales del segundo caso, haciendo que sufrieran stress físico por el efecto del transporte, pero no stress emocional o psicológico producto del embarque y el desembarque (Grandin, 1997).

Trunkfield y Broom (1990) registraron incrementos importantes en los valores de cortisol en sangre durante las primeras 2 horas de transporte, sugiriendo que los animales se encuentran estresados durante las fases iniciales del mismo. Villaroel et al (2003) también indicaron que en viajes relativamente de corta duración (menos de 4 horas), los niveles de cortisol en sangre fueron altos en las primeras 2 horas de transporte, determinando que luego de ese período inicial los animales se habituaron a la nueva situación.

Numerosos autores han reportado incrementos en los niveles de CPK en sangre, luego del transporte (Grasso et al., 1989; Groth et al., 1977; Van de Water et al., 2003; Villaroel et al., 2003). Ello podría indicar posibles traumas ocasionados por la carga y descarga, por el transporte mismo debido al esfuerzo que los animales deben hacer para mantenerse en pie

y no contactarse, así como por la posible interacción que generalmente ocurre entre ellos (Anderson et al., 1976; Lefebvre et al., 1996).

Como consecuencia del transporte también se han reportado incrementos en la concentración de ácidos grasos libres, beta hidroxibutirato y glucosa en sangre, interpretándolo tanto como incrementos en la actividad metabólica (movilización de reservas corporales para satisfacer los requerimientos de energía (Warriss et al., 1995) o como consecuencia de una situación de estrés agudo (efecto de las catecolaminas sobre el tejido adiposo) (Shaw y Tume, 1992).

Todos estos factores deben considerarse al momento de redactar y/o actualizar legislaciones o recomendaciones acerca de la duración máxima del transporte en cada especie y acerca de la conveniencia de realizar paradas de descanso, etc.. En animales que provienen de condiciones extensivas, cada parada puede agregar un stress adicional, además de aumentar las probabilidades de transmisión de enfermedades (Grandin, 1997).

Algunos autores afirman que los factores más importantes que determinan el bienestar de los animales en el transporte son el diseño del vehículo, la densidad de animales, la ventilación, la calidad de la conducción del vehículo y la calidad o características de la ruta. (Broom, 2003; Hartung, 2003; Tarrant and Grandin, 1993).

El clima o microclima logrado en el interior del medio de transporte es un factor importante que influye en el bienestar de los animales. La ventilación y la calidad del aire deben ser controlados: temperatura, humedad relativa, gases contaminantes, otros contaminantes (Wikner et al., 2003). En este sentido, la densidad de animales en el vehículo es un parámetro importante que presenta repercusiones económicas de relevancia. Altas densidades hacen que los animales experimenten una fatiga adicional durante el transporte ya que no tienen espacio suficiente para girar, etc., obligándolos a realizar cambios de postura en forma frecuente (Tarrant et al., 1988). También aumentan las peleas y adicionalmente la temperatura del vehículo se eleva, pudiendo ocasionar stress térmico. Por otra parte, una baja densidad incrementa las probabilidades de golpes por pérdidas de equilibrio a causa de movimiento del vehículo. Es así que la densidad tiene efecto sobre el BA y la calidad de la canal y de la carne. Se deben definir las densidades óptimas para cada especie y categoría. Estas variarán según la época, el temperamento de los animales y la duración del transporte (Directiva 85/29/CE del 29 de junio de 1995; FAWC, 1992); (EUROPEAN COMMISSION, 2002).

La calidad de la conducción así como el estado de las rutas también son factores importantes. Hay estudios que demuestran que las vibraciones ocurridas durante el transporte comprometen el bienestar de los animales jóvenes (Van de Water et al., 2003). Al

respecto, otros autores sostienen que el factor más estresante (determinado por el mayor incremento de cortisol en plasma), es el movimiento del vehículo, comparado con los procesos de carga y descarga, y la espera en frigorífico (Kenny y Tarrant, 1987). Ruiz de la Torre et al. (2001) demostraron que corderos que son transportados en rutas en buen estado presentan una menor alteración del ritmo cardíaco, menores concentraciones de cortisol en plasma y valores de pH más bajos luego de 10 y 12 horas de viaje, comparados con aquellos animales que viajaban por rutas en mal estado.

2.2.6.2. Espera previa al sacrificio

La espera en matadero es un factor fundamental ya que durante la misma se generan diversas situaciones estresantes para los animales (Marahrens et al, 2003). No existe un acuerdo acerca de la duración ideal del ayuno pre faena. Este dependerá de la duración y las condiciones del transporte, del vehículo, la alimentación, etc.. El principal problema reside en que generalmente no se conoce el tiempo que los animales esperarán en la planta de faena, por lo cual no se podría planificar las horas de ayuno exactamente (Manteca et al., 2001). El ayuno previo a la faena presenta ciertas ventajas en lo que tiene que ver con la facilidad operativa y la inocuidad alimentaria. Sin embargo, es importante considerar que ayunos muy prolongados pueden provocar efectos muy negativos en el BA debido a las sensaciones de hambre, podrían aumentar la incidencia de carnes de baja calidad y disminuir el peso de la canal.

Algunos autores consideran que la espera permite la rehidratación de los animales así como el descanso y recuperación del probable cansancio ocasionado por el viaje, permitiendo de esta manera la recuperación de los niveles de glucógeno del músculo (Mounier et al., 2006; Warriss et al., 1984). Por otra parte, diversos autores afirman que la espera en sí constituye un factor negativo, no permitiendo que los animales se recuperan de la privación de agua y alimento (Jarvis et al., 1996) por lo cual largas esperas se asocian con una depresión en la calidad de la canal y la carne. Estas opiniones contradictorias podría explicarse por diversos factores entre los que se destacan: duración y características del transporte y de las esperas evaluadas, historia de los animales (genotipo, temperamento, alimentación), condiciones de manejo e infraestructura del matadero, entre otras. Gallo et al. (2003) reportaron que mayores tiempos de espera (3, 6, 12 y 24 horas luego de 3 y 16 horas de viaje) implicaban un deterioro en la calidad de la carne de novillos. Por otra parte, Ferguson et al. (2007) no encontraron diferencias en la terneza de carne proveniente de animales que estuvieron en corrales de espera durante 3 y 18 horas.

Respecto a algunos indicadores fisiológicos, Tadich et al. (2005) determinaron incrementos de CPK luego del transporte (0, 3 y 16 horas), pero no luego de la espera en corrales, ante

diferentes combinaciones de las mencionadas horas de transporte y espera en corrales de 0, 3, 12, 16 y 24 horas.

En relación al posible esfuerzo metabólico que deben hacer los animales ante situaciones de privación de alimentos, se destaca que Jarvis et al (1996) registraron concentraciones de AGL mayores en aquellos animales que pasaron más de 16 horas en corrales de espera, comparados con animales de 5 horas de espera. También Cockram y Corley (1991) han reportado mayores concentraciones de AGL en sangre de animales que han pasado la noche en corrales de espera, en comparación a los que fueron faenados el mismo día de arribo a planta.

Sin embargo, es muy difícil determinar el punto de quiebre o umbral o en que esos valores comienzan a comprometer el BA. A su vez, es muy difícil comparar resultados de diferentes experimentos, considerando las diferentes situaciones e historias productivas de los animales.

A la luz de la información diferente y en muchos casos contradictoria, tanto respecto a transporte como al tiempo de espera previo a la faena, se considera que los resultados productivos, fisiológicos y comportamentales que pretenden cuantificar el BA, deben interpretarse en el marco del diseño y condiciones de cada experimento o situación. A su vez dichos resultados requieren de una interpretación integral, acorde al contexto productivo o industrial en que han sido generados.

2.2.7. Temperamento

Además del carácter del agente estresante, existen diferencias individuales (experiencia y factores genéticos) que afectan la respuesta neuroendocrina y neuroquímica frente al stress, las que también tienen influencia en la respuesta del sistema inmunológico frente a estos desafíos (Anisman, 2002). Las diferencias individuales en lo que tiene que ver con respuestas fisiológicas y comportamentales ante situaciones de estrés, han sido demostradas por diversos autores (Grandin, 1997; Le Neindre et al., 1995).

El temperamento se define como el comportamiento del animal en respuesta al manejo (Burrow, 1997). Los animales de temperamento negativo, más agresivos o nerviosos son más propensos a sufrir incidentes, inicialmente reaccionan en forma más enérgica a situaciones novedosas, son más propensos a vocalizar ante pequeñas distracciones como sombras o reflejos y son más agresivos con sus congéneres, habiéndose registrado esto tanto en establecimientos, en ferias ganaderas, como en plantas de faena. Cuando se practican manejos no dolorosos en forma reiterada a animales excitables y calmos, los primeros muestran una mayor dificultad para adaptarse a ellos. Asimismo, el manejo

inadecuado de animales con temperamento excitable, será más perjudicial que cuando se realiza con animales calmos (Grandin, 1997).

El temperamento presentaría una influencia sustancial sobre la rentabilidad de las empresas de producción de carne en condiciones extensivas, debido a su efecto en el incremento de los costos de producción (Anon. 1988), posiblemente a través de la relación temperamento - crecimiento o engorde (Tulloh, 1961) y a través de la relación temperamento - calidad de canal y carne (Burrow 1997; Fordyce et al., 1988 b). Los animales de temperamento más excitable serán más susceptibles al estrés generado ante situaciones rutinarias de manejo tales como movimientos de potrero, procedimientos sanitarios, así como también ante situaciones novedosas tales como el embarque y desembarque, transporte, la espera en corrales de matadero (Lensink et al., 2000). Dicho estrés previo a la faena, reduciría el contenido del glucógeno del músculo del animal vivo (mayor gasto energético debido a privación de alimentos, miedo, comportamientos agresivos, montas, peleas, etc.) lo cual produciría un pH último más elevado (Gregory y Grandin, 1998). De esta forma se verían afectadas diversas características deseables de calidad de carne, tales como la terneza (Purchas et al., 1996) y el color. Selye (1936) sostiene que el estrés, sea cual sea su factor generador (físico o psicológico) resulta en una descarga hormonal que indirectamente implica la remoción/destrucción de glucógeno. Si un animal de temperamento excitable es deficiente en la producción de corticosteroides para la recuperación de esos niveles de glucógeno, se mantendrán esos bajos niveles crónicos de glucógeno. Es así que cuando estos animales son sometidos a un factor adicional de estrés estarán más propensos que un animal calmo o de temperamento normal, a presentar pH elevados. Según (Bray et al, 1989) los factores estresantes son aditivos, por lo que la ocurrencia de factores estresantes múltiples en las etapas previo a la faena, tendrían un efecto mayor sobre el pH y otros indicadores de calidad de carne, que cuando ocurren en forma aislada.

2.2.7.1. Razas y Temperamento

Basado en una revisión sobre temperamento en bovinos para carne, Burrow (1997) sostiene que sin excepción, las razas con componentes *Bos indicus* son más excitables, temperamentales y difíciles de manejar en condiciones extensivas, que las razas *Bos Taurus* (Burrow y Corbet, 2000; Elder et al., 1980; Fordyce et al., 1988 a; Hearnshaw et al., 1979; Hearnshaw y Morris, 1984; Powell y Reid, 1982; Voisinet et al., 1997). A pesar de que estas diferencias podrían en parte deberse a factores ambientales, las diferencias genéticas en cuanto a docilidad o excitabilidad del ganado bovino, han sido demostradas (Manteca y de la Torre, 1996). Algunos autores han reportado incluso que la raza Hereford sería la más dócil dentro de las razas británicas (Stricklin et al., 1980; Tulloh, 1961).

2.2.7.2. Determinación del temperamento

Muchos autores han utilizado y adaptado diversas metodologías para la determinación del temperamento en bovinos (Burrow et al., 1988; Grandin y Deesing, 1998; Hearnshaw et al., 1979; Sato, 1981). Una herramienta para medir temperamento debe ser confiable, repetible y deberá estar vinculada a la respuesta individual frente al estrés. Se han utilizado diversas técnicas, siendo muchas de ellas subjetivas, lo cual incrementaría las posibilidades de que exista error humano y/o sesgo en la determinación del temperamento de los animales (Curley et al., 2006).

Burrow (1997) indica que los test para la determinación del temperamento pueden clasificarse en dos categorías: "restrictivos" (*restrained*) y "no restrictivos" (*non-restrained*). En la primera categoría se registra el comportamiento del animal frente a la presencia humana, en condiciones de movimiento restringido o controlado. Estos tests generalmente se basan en apreciaciones subjetivas sobre la resistencia que manifiestan los animales ante esa restricción física. En la segunda categoría el animal se encuentra libre en un área relativamente extensa y se computa su comportamiento en relación a la respuesta al miedo frente al ser humano u a otro factor determinado. Dentro de dichas categorías y a los efectos del presente trabajo, se destacan los siguientes tests:

Restrictivos

- Crush score (CS): se registra el comportamiento del animal cuando es sometido a una situación de encierro /cepo, balanza), utilizándose una escala subjetiva que va desde 1 (animal muy calmo) a 5 (animal muy excitable).

No restrictivos

- Flight-time o Flight Speed (FT): es el tiempo que lleva a un animal recorrer una distancia estipulada de antemano, una vez que es liberado de la situación de encierro. Según Burrow et al. (1988) es una herramienta objetiva, segura y rápida que puede ser implementada fácilmente a nivel de producción.
- Exit speed (ES) es la velocidad con que los animales salen de la situación de encierro, por lo que estaría midiendo el mismo comportamiento que la herramienta anterior, pero medida en una escala ordinal. 1 = caminando, 2 = trotando, y 3 = corriendo.
- Zona de Fuga (Flight zone): es el espacio personal del animal y cuando una persona entra al mismo, el animal tiende a alejarse. Dicho test puede realizarse a nivel individual o a nivel grupal.

Otras medidas

Según Grandin et al. (1996) la ubicación del rulo en espiral de la cara en bovinos, podría ser un indicador de su temperamento. Esta relación podría explicarse por el hecho de que los patrones de crecimiento del pelo se forman en el feto en forma simultánea a la formación del cerebro (Smith y Gong, 1974). Cuando el rulo de la cara se sitúa sobre el nivel de los ojos, el animal tendrá un temperamento más excitable y estará más agitado durante el manejo, cuando está al nivel de los ojos será más calmo y cuando el rulo está por debajo del nivel de los ojos, el temperamento será aún más calmo. Si bien los resultados con el uso de esta técnica no son contundentes, parecería ser una herramienta útil para la estimación del temperamento en aquellos animales que nunca han tenido contacto con el hombre (Randle, 1998).

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CAPÍTULO III - OBJETIVOS

CAPÍTULO III - OBJETIVOS

El presente trabajo de tesis comprende 2 experimentos realizados en las condiciones de producción del Uruguay, con el objetivo de evaluar el efecto de diferentes sistemas de alimentación y manejo pre faena, sobre el Bienestar animal y la calidad de carne de novillos.

Objetivos específicos Experimento 1:

- ❖ Determinar el efecto de diferentes sistemas de alimentación con niveles incrementales de grano en la dieta, sobre la ganancia de peso vivo de los animales y días a la faena
- ❖ Determinar el efecto de dichos sistemas de alimentación sobre el comportamiento de los animales en fase de producción
- ❖ Determinar el efecto de dichos sistemas de alimentación, sobre la calidad de la canal y de la carne
- ❖ Evaluar las respuestas individuales de los animales, ante diferentes situaciones de producción y /o estrés
- ❖ Evaluar el efecto del temperamento sobre la calidad de la carne

Objetivos específicos Experimento 2:

- ❖ Determinar el efecto del transporte y de dos tiempos contrastantes de espera, sobre diferentes indicadores fisiológicos relativos al Bienestar animal
- ❖ Determinar el efecto de dos sistemas de alimentación y dos tiempos contrastantes de espera sobre el comportamiento de los animales en corrales de matadero
- ❖ Determinar el efecto de dos sistemas de alimentación y dos tiempos contrastante de espera sobre la calidad de la canal y de la carne
- ❖ Evaluar las respuestas individuales de los animales ante diferentes situaciones de estrés previas a la faena
- ❖ Evaluar el efecto del temperamento sobre la calidad de la canal y de la carne

Los resultados se presentan en cuatro artículos científicos:

Experimento 1:

- ❖ Artículo 1: Effects of feeding strategies including different proportion of pasture and concentrate, on carcass and meat quality traits in Uruguayan steers
- ❖ Artículo 2: Effect of different finishing strategies on animal welfare and beef quality in Uruguay

Experimento 2:

- ❖ Artículo 3: Finishing diet, lairage time and temperament effects on carcass and meat quality traits in Uruguayan steers
- ❖ Artículo 4: Animal welfare related to temperament and different pre slaughter procedures in Uruguay

CAPÍTULO IV - ARTÍCULO 1

CAPÍTULO IV - Effects of feeding strategies including different proportion of pasture and concentrate, on carcass and meat quality traits in Uruguayan steers²

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Abstract

Eighty four steers were randomly assigned to three pasture treatments with increasing levels of grain (T1: 0%; T2: 0.6%; T3: 1.2% of live weight) and to an *ad libitum* concentrate treatment, T4, to study the effects on carcass and meat quality. Animals were slaughtered with 500 kg of average live weight per treatment. Average daily gain increased with increasing levels of energy, determining different slaughter dates. Intermediate treatments showed higher carcass weight than T1. T4 and T3 had a higher weight of valuable cuts than T1 and T4. Pistolas from T4 had a higher fat proportion and lower bone percentage. Increasing levels of energy in diet decreased fat yellowness. After 20 days of aging, T4 had the lowest muscle a^* values and shear force was higher for T4 than for T1. With pasture finishing strategy, no adverse effects on meat quality were detected and tenderness was enhanced.

Keywords: diet, beef, carcass traits, meat quality

1. Introduction

Uruguay is a primary meat exporter, being the 7th country in the world in volume, on which its economy is highly dependent. Meat production systems are mainly based on native pastures but the use of intensive systems is growing in order to improve animal performance and carcass and meat quality traits, as well as to increase the number of animals in the system. These intensive systems include a wide range of feeding alternatives between pasture and concentrate utilization. The use of concentrates in pastures during limited periods or for finishing purposes is becoming more common. This could involve many differences in terms of carcass and meat quality, which require to be studied. The challenge is to produce a finishing strategy to improve the product without modifying the peculiar characteristics acquired during extensive grazing conditions (low-cost production and healthy

² Artículo publicado en la Revista Meat Science, 2008. *Meat Science*, 80: 753-760. Ver Anexo.

meat for human consumption), and without compromising neither animal welfare status nor the environment. Finishing strategies in cattle have been extensively studied over the years with varied results on carcass traits and meat quality. Many studies have traditionally discounted meat tenderness from extensive systems as compared to concentrate-fed beef (Christall, 1994) and also colour (French et al., 2000). In many of these experiments, dietary effects were confused with animal age, pre-slaughter growth rate or carcass weight/fatness ratio at slaughter (French et al., 2001). Many other studies reported no differences in quality and acceptability in meat from animals finished on pastures or concentrates ([French et al., 2001; Mandell et al., 1998; Vestergaard et al., 2000]). Other authors reported that forage-fed beef had equal or superior eating qualities including tenderness, than concentrate-fed beef (Oltjen et al., 1971; Realini et al., 2004). This variability in results indicates that further research is necessary to establish the reasons for these differences.

The objective of this experiment was to evaluate the effect of different diets, including pastures with increasing levels of grain as supplement and a concentrate diet, on carcass and meat quality traits in Uruguayan steers.

2. Materials and methods

The study was run by the National Institute of Agricultural Research, at INIA La Estanzuela Research Station, Uruguay (Latitude 34°20'18" South, Longitude 57°41'24" West), over a period of 8 months (from September 2005 to April 2006).

2.1. Animals and diets

Eighty four Hereford steers with a common origin and background on pasture, (1.5 years old and 391 kg of live weight on average), were randomly assigned to one of the following diets:

- (a) T1 – pasture: offered at 4% of live weight (LW).
- (b) T2 – pasture: offered at 3% of LW and grain (0.6% of LW).
- (c) T3 – pasture: offered at 3% of LW and grain (1.2% of LW).
- (d) T4 – concentrate and good quality alfalfa hay, offered *ad libitum*.

Corn grain was used in treatments 2 and 3. The concentrate diet was composed of 85% corn, 13% sunflower, 0.98% urea, and salt, minerals and vitamins. Alfalfa hay was chopped (2–3 cm) and provided with the concentrate. The pasture, composed of alfalfa (*Medicago sativa*), white clover (*Trifolium repens*) and fescue (*Festuca arundinacea*), was divided into nine plots by electric fencing, in which T1, T2 and T3 were released (three repetitions of seven animals per treatment). The intensive feed treatment (T4) was located in three open-

air plots on concrete flooring. Animals from all treatments had *ad libitum* access to water and mineral salts. Chemical composition of feeds offered, are reported in Table 1.

Table 1. Chemical composition of feeds offered.

	OMD (%)	CP (%)	ADF (%)	NDF (%)	A (%)
Pasture	61.23	17.17	42.11	53.83	11.88
Corn grain	82.19	9.18	6.53	21.59	3.04
Concentrate	85.83	12.43	12.50	36.34	2.88
Alfalfa hay	57.03	23.26	33.40	39.54	11.11

OMD: organic matter digestibility, CP: crude protein, ADF: acid detergent fibre, NDF: neutral detergent fibre, A: ash

Pasture height, availability and quality were registered for each plot in 7-day grazing periods (pre and post grazing). The same data was collected for the pasture remaining in each plot. Botanical composition was registered throughout the experiment. Pasture results are not presented in this paper.

Animals were weighed early in the morning without previous fasting every 14 days. The grazing area for pasture-based treatments for the subsequent 7-days was calculated according to live weight and available dry matter. The grazing system was planned in order to avoid over-grazing and the corn grain was provided early in the morning in T2 and T3. Animals from T4 received the concentrate twice a day (morning and afternoon).

Ultrasound measurements were taken at intervals of 28 days, registering ribeye area and fat depth at the *Longissimus dorsi* muscle between the 12th and 13th rib, and fat depth in the rump area.

2.2. Slaughter and sampling procedures

Animals were slaughtered by humanitarian procedures in a commercial abattoir when they reached an average of 500 kg of LW in each treatment and at least 6 mm of fat covering (determined by ultrasound technique). Growth rates differed for all treatments and concentrate-fed animals (T4) were slaughtered on December 10th, 2005; followed by steers from T3 (December 27th, 2005), from T2 (January 24th, 2006) and from T1 on April 4th, 2006.

Carcasses were graded using the European Union (EU) beef carcass Classification System (EU Legislation, 1991) and the Uruguayan Grading System as specified by I.N.A.C. (1997). Both systems are based on conformation and fatness scores. Carcass conformation was based on a visual assessment of muscle mass development, with lower numbers indicating better conformation (1 = good muscle development and 6 = poor muscle development). The

conformation score system for Uruguay is : I(1), N(2), A(3), C(4), U(5), R(6), and for the EU: S(1), E(2), U(3), R(4), O(5), P(6).

Fat finishing was based on the amount and distribution of subcutaneous fat, using a five grade scale and also subclasses, where lower numbers indicate lack of fat cover and higher numbers, excessive covering. The scores used by Uruguay are: 0, 1, 2, 3, 4, and by the EU: 1, 2, 3, 4, 5.

The Uruguayan system also includes a dentition scale from 1 to 4, based on the number of teeth. A lower score indicates a younger animal (1: *yearling steer* – without permanent incisors, 2: *young steer* – two to four permanent incisors, 3: *young steer with six teeth*, and 4: *steer* – eight permanent incisors).

Hot carcass weight (HCW) was registered. Carcass pH and temperature were measured at 1, 3 and 24 h post mortem (pm) at the *Longissimus dorsi* (LD) muscle between 12th and 13th rib, using a thermometer (Barnant 115) with Type E thermocouple and pH meter (Orion 210A) with gel device. At 24 h pm carcasses were ribbed between 5th and 6th rib, obtaining primal cuts. Subcutaneous fat thickness and fat colour were recorded at this time. The former was registered at the L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) colour space, using a colorimeter (Minolta C10) with an 8 mm diameter measurement area.

The pistola cut was prepared from the hindquarter by the removal of the thin flank, lateral portion ribs and a portion of the navel end brisket. A cut was made from the superficial inguinal lymph node, separating the *Rectus abdominus* and following the hip contour, running parallel to body of the vertebra and the *Longissimus dorsi* muscle (eye muscle) to the specified ribs. The fabrication process was carried out according to a European commercial standard (United Kingdom). From the pistola cut, 7 boneless cuts were obtained (Striploin, Tenderloin, Rump, Topside, Silverside, Knuckle, Tail of rump), and the weights of trimmings and bones were recorded. Retail cuts were weighed and retail yield was calculated (Rump and Loin³, pistola cut/hindquarter, 7 cuts/pistola cut; R&L/pistola cut, others). Muscle, fat and bone percentages were calculated from the retail cuts, using the UK commercial standard (5% fat cover).

2.3. Meat quality

Two steaks (2.54 cm thickness) were vacuum packaged individually, transported to INIA Tacuarembó Meat Laboratory, and aged for 7 and 20 days at 2–4 °C. Meat colour, toughness and cooking loss were measured after each aging period.

³ Rump and Loin (R&L): Striploin, tenderloin and rump

Instrumental colour. Meat bags were opened and the exudation on the steak surface was removed with a paper towel. Muscle colour was measured on the LD after seven and twenty days of aging at the L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) colour space, using a colorimeter (Minolta C10) with an 8 mm diameter measurement area, after one hour of blooming. Values were registered from three different locations on the upper side of the steaks in order to obtain a representative average value of the meat colour.

Shear force. The LD steaks were placed inside polyethylene bags and cooked in water bath until an internal temperature of 70 °C was achieved, using a Barnant 115 thermometer with type E thermocouple. Six cores, 1.27 cm diameter, were removed from each steak parallel to the muscle fiber orientation. Shear force measurement (SF) was obtained for each core using Warner Bratzler (Model D 2000), and an average value was calculated for each steak.

Cooking loss. This was determined by the difference between the weight of the steak after cooking and its pre-cooked weight, shown as a percentage.

2.4. Statistical analysis

Exploratory analyses were performed with the Statgraphics and SAS programmes. Outliers were evaluated and eliminated. The relationship among variables was evaluated using Pearson correlations. A principal component analysis (PCA) was performed to describe the relationship between meat quality traits.

The statistical model applied was

$$y = t + b_1 I_w + b_2 F_w + e$$

where t is treatment; I_w , initial live weight; F_w , final live weight and e is the error.

Final and initial live weights were used as covariates and the model included treatment as a fixed effect. Interactions between final weight and treatment were eliminated, as they had no significant effect on the results obtained. Results were analysed by the GLM SAS procedure. LSM Means and differences between treatments were estimated.

With the exception of final live weight, conformation and finishing degree, all variables were adjusted for initial and final live weight.

3. Results and discussion

3.1. Animal performance and carcass traits

Concentrate-fed animals (T4) had the highest average daily gain (ADG), which increased with the level of energy in diet (Table 2). In general, when pastures and grains or concentrates are offered *ad libitum*, ADG is higher in concentrate-fed cattle, relative to grass-

fed animals (French et al., 2000) and (French et al., 2001). Thus, concentrate-fed animals are younger when compared at a specific bodyweight or back-fat thickness. In our experiment, the use of high-energy diets even when not *ad libitum* (T2 and T3), increased ADG and decreased days to slaughtering, involving different slaughter dates and consequently, animal age. Similar results were found by Gil and Huertas (2001) when comparing animals from grain diets and high quality pastures. In our experiment, this produced a higher percentage of 4-teeth animals in the pasture treatment (T1). Forty percent of the former were 2-teeth while in other treatments, animals with this dentition were more than 80 %.

Table 2. Effect of finishing strategy on daily gain, final live weight, hot carcass weight, pistola cut weight and boneless cuts weight.

General mean, residual typical deviation (σ_r), coefficient of variation (CV), difference between treatments, standard error (SE) and *p* value.

Characteristic	General mean	σ_r	CV	Contrast	Difference	SE	<i>p</i> value
Daily gain (Kg)	1.18	0.045	3.79	T1-T2	-0.3237	0.014	<.0001
				T1-T3	0.5608	0.014	<.0001
				T1-T4	-0.8807	0.018	<.0001
				T2-T3	-0.2371	0.014	<.0001
				T2-T4	-0.5570	0.018	<.0001
				T3-T4	-0.3199	0.018	<.0001
Final live wt (kg)	506.8	21.7	4.3	T1-T2	5.2928	6.967	0.4499
				T1-T3	-0.3927	6.949	0.9551
				T1-T4	-41.5424	7.078	<.0001
				T2-T3	-5.6855	6.885	0.4116
				T2-T4	-46.8352	6.953	<.0001
				T3-T4	-41.1497	7.001	<.0001
Hot carcass wt (kg)	267.99	6.835	2.55	T1-T2	-7.0014	2.204	0.0022
				T1-T3	-7.4566	2.190	0.0011
				T1-T4	-0.4433	2.706	0.8703
				T2-T3	-0.4552	2.180	0.8352
				T2-T4	6.5581	2.790	0.0215
				T3-T4	7.0133	2.678	0.0107
Pistola cut wt (Kg)	64.34	2.108	3.28	T1-T2	-1.0948	0.680	0.1118
				T1-T3	-2.5919	0.676	0.0003
				T1-T4	-0.5870	0.835	0.4843
				T2-T3	-1.4970	0.673	0.0291
				T2-T4	0.5078	0.861	0.5571
				T3-T4	2.0049	0.826	0.0177
7 boneless cuts wt (Kg)	34.54	1.911	5.53	T1-T2	-0.6771	0.616	0.2757
				T1-T3	-1.5746	0.620	0.0133
				T1-T4	0.8359	0.760	0.2753
				T2-T3	-0.8974	0.618	0.1508
				T2-T4	1.5130	0.784	0.0576
				T3-T4	2.4105	0.749	0.0020

T1: Pastures, T2: Pastures + 0.6 % grain of LW, T3: Pastures + 1.2 % grain of LW, T4: concentrate *ad libitum* plus alfalfa hay

Concentrate-fed animals had higher final live weights (FLW) at slaughter (Table 2). No differences were found among other treatments in this variable.

Intermediate treatments (T2 and T3), had the higher hot carcass weight and T3 had the heaviest pistola cut. This information is consistent with that reported by Vaz Martins et al. (2003) when comparing pistola cut weight from pasture treatment and pasture plus grain (offered at 0.7% and 1.5% of LW), where treatment with grain offered at 1.5% of LW, showed the highest pistola cut weight. In this experiment, no differences were found among T1, T2 and T4 in pistola cut weight.

T3 also had higher weight of seven boneless cuts when compared with the pasture treatment (T1) and the concentrate one (T4) (Table 2). Butterfield (1974) indicated that meat yield decreased as carcass weight increased (probably due to higher fat contents). However, in our experiment there were no differences in valuable cuts yield (meat yield), between T3 and the other treatments.

R&L represent most of the commercial value of the carcass. Some markets require a certain weight for each cut. In this experiment, R&L weight and the retail yield of the R&L (R&L/hindquarter, R&L/pistola cut) did not show relevant differences between treatments.

Finishing strategies had an effect on carcass conformation and fat grading, according to the European and Uruguayan classification systems. According to the European Union Scheme (EU), carcasses from T1 had a poorer conformation than those from the other treatments, which did not differ among them. In pasture-fed animals, 45% of the carcass had an "O" conformation, while 55% were "R". Most of the other treatment carcasses were classified as "R". High-energy diets during the finishing period, significantly improve carcass conformation (O'Ferrall and Keane, 1990).

Carcasses from T4 had higher fatness scores according to the EU system, 95% showing a score equal or higher than 4 (Fig. 1). Only 30% of carcasses from T2 reached this score, and those from only pastures (T1) did not reach this figure. Eighty five percent of the former had a score of three or less. Results for only pasture-fed carcasses are similar to those reported by Montossi and Sañudo (2007) when evaluating Uruguayan steers with different ages (2 and 3 years-old).

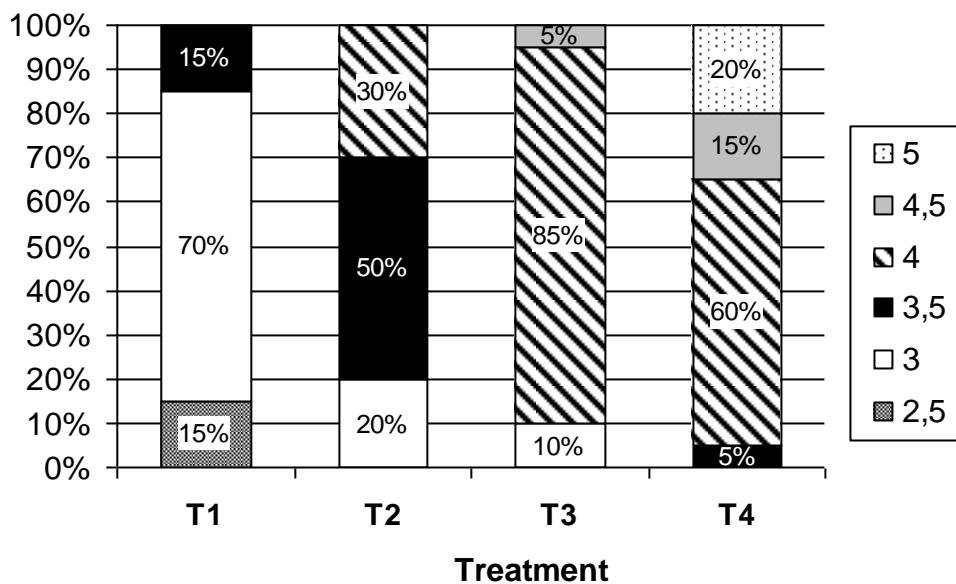


Figure 1. Results of applying the EU Fat Grading system, by treatment.

0 = poorer finishing, 5= excessive fat cover.

T1: Pastures, T2: Pastures + 0.6 % grain of LW, T3: Pastures + 1.2 % grain of LW, T4: concentrate ad libitum plus alfalfa hay

Ultrasonic final fat cover did not show differences between treatments. Neither did subcutaneous fat (fat thickness) on the carcasses, show any difference between treatments, giving a general mean of 10.36, a residual typical deviation (σ_r) of 2.81 and 27.1 of coefficient of variation (CV). According to Robelin et al. (1979), the chronological order in fat deposition is intermuscular, internal, subcutaneous and intramuscular. This could explain differences obtained in fat percentage but not in fat cover. The finishing period in this experiment could have been enough to establish intermuscular and internal fat differences but not subcutaneous fat differences.

Muscle percentage did not show differences between treatments when pistola cut composition was evaluated, although many studies reported that a higher concentrate level during finishing periods leads to a lower proportion of muscle and bone in the carcass, along with a higher percentage of fat (Keane et al., 1989).

Pistolas from treatments with high-energy levels in diet (T3 and T4) had higher fat percentage than pistolas from T1 and T2 (Table 3). Similar results were obtained by other authors, reporting that fat proportion rose with concentrate-based diets (Micol, 1993). A fast growth rate caused by a high plane of nutrition, can lead to an earlier onset of the fattening phase of growth (Lawrie, 1998). In fact, in this experiment, ADG had a positive and significant correlation ($p < 0.001$, $r = 0.62$) with pistola fat percentage. Similar results were

found by Cerdeño et al. (2006). Fat ratio changed as long as ADG increased, due to the different planes of energy in the diet.

Table 3. Fat and bone ratio in pistola cut.

General mean, residual typical deviation (σ_r), coefficient of variation (CV), difference between treatments, standard error (SE) and p value.

Characteristic	General mean	σ_r	CV	Contrast	Difference	SE	p value
Fat* (%)	0.1040	0.017	16.7	T1 – T2	0.0025	0.006	0.661
				T1 – T3	-0.0203	0.006	0.001
				T1 – T4	-0.0265	0.007	0.000
				T2 – T3	-0.0229	0.006	0.000
				T2 – T4	-0.0291	0.007	0.000
				T3 – T4	-0.0062	0.007	0.364
Bone (%)	0.2144	0.013	5.88	T1 – T2	-0.0016	0.004	0.697
				T1 – T3	0.0038	0.004	0.351
				T1 – T4	0.0233	0.005	<0.000
				T2 – T3	0.0054	0.004	0.185
				T2 – T4	0.0250	0.005	<0.000
				T3 – T4	0.0196	0.005	0.000

*pistola fat + kidney fat

T1: Pastures, T2: Pastures + 0.6 % grain of LW, T3: Pastures + 1.2 % grain of LW, T4: concentrate ad libitum plus alfalfa hay

Pistola bone proportion did not show differences among T1, T2 and T3. However, all of them had higher bone percentages than the concentrate treatment (Table 3). Carcass composition is weight-dependent and largely influenced by age or nutritional regime. Other authors reported that even animals compared at a common weight will differ greatly in form and composition when they are on different levels of nutrition. Differences in carcass composition are mainly related to the differences in the energy density of the diet and, in the end, to the total energy consumed (Cerdeño et al., 2006).

According to Sañudo (1997) higher carcass weight produces higher muscle thickness and fat deposits, which means the carcass and all its components have greater dimensions. In a relative value, higher carcass weight implies higher fat tissue and late mature zones, lower bone tissue and early mature components, and a more or less clear stabilization of muscle tissue and other isometric zones (those whose growth is proportional to the total growth).

3.2. Fat and meat quality

Grass-based treatments (T1, T2 and T3) had higher pH values than T4 (Table 4) at 1 and 3 h pm. Similar results were found by French et al. (2001), who reported that LD from pasture-fed animals had higher pH at 4, 5, 6, 7, and 8 h post slaughter when compared with LD from concentrate-fed cattle. This could be related to differences in muscle glycogen content,

higher in animals fed with high-energy diets; and also to differences in stress susceptibility in pre-slaughter handling, higher in extensively reared animals. In the same way, pH values at 24 h pm were higher in carcasses from T1 than carcasses from T2 and T4 (Table 4). The pH decline depends on muscle glycogen concentration. Daly et al. (2002) showed that higher initial muscle glycogen concentration resulted in a faster rate of pH decline. The two major reasons for suboptimal glycogen levels at slaughter are a poor nutritional plane on farm (Pethick and Rowe, 1996) or excessive glycogen losses from stress during transport and lairage. According to Immonen et al. (2000) glycogen levels in muscle increase with metabolisable energy intake. These glycogen stores can then act as a buffer in the pre-slaughter period. These authors reported that the effect of a high-energy diet on the muscle content was reflected all the way to ultimate pH values and residual glycogen concentrations. Even a short finishing period of 2 weeks with a concentrate-based high-energy diet, was well worth applying because its clearly protective effects were directly against glycogen depletion and elevation of pH. Muir et al. (1998) also reported that grass-fed steers had higher ultimate pH values than grain-fed ones, and they suggested that animals from pasture diets are more susceptible to pre-slaughter stress, so they could have had less glycogen content in muscle and consequently higher final and intermediates pH values than grain-fed steers. Feedlot steers are more used to penning and handling and would be less likely to suffer glycogen depletion during the pre-slaughter period. In this experiment, all animals were used to handling and penning because of frequent weighing, and a strict protocol of Good Management Practices was followed during the whole process. However, animals from T1 had less daily contact with humans. Other authors did not find differences in ultimate muscle pH in grain and grass-finished cattle (French et al., 2000; French et al., 2001; Morris et al., 1997). These contradictory results could be partially explained by factors such as breed, animal temperament, management practices or just previous animal experience in handling.

In spite of the differences in initial and final pH values in our experiment, all were within the normal range and without incidence of dark cutting beef.

Table 4. Carcass pH at 1, 3 and 24 hours *post mortem*.

General mean, residual typical deviation (σ_r), coefficient of variation (CV), differences between treatments, standard error (SE) and *p* value.

Characteristic	General mean	σ_r	CV	Contrast	Difference	SE	<i>p</i> value
pH 1	6.48	0.121	1.86	T1-T2	-0.0693	0.0389	0.0790
				T1-T3	0.0570	0.0386	0.1441
				T1-T4	0.3602	0.0477	<.0001
				T2-T3	0.1263	0.0384	0.0016
				T2-T4	0.4294	0.0492	<.0001
				T3-T4	0.3031	0.0472	<.0001
pH 3	6.17	0.128	2.07	T1-T2	0.0037	0.0412	0.9293
				T1-T3	0.1108	0.0410	0.0086
				T1-T4	0.2688	0.0506	<.0001
				T2-T3	0.1071	0.0408	0.0106
				T2-T4	0.2651	0.0522	<.0001
				T3-T4	0.1580	0.0501	0.0024
pH 24	5.61	0.117	2.08	T1-T2	0.1661	0.0377	<.0001
				T1-T3	-0.0245	0.0374	0.5151
				T1-T4	0.2348	0.0462	<.0001
				T2-T3	-0.1906	0.0372	<.0001
				T2-T4	0.0687	0.0477	0.1540
				T3-T4	0.2592	0.0458	<.0001

T1: Pastures, T2: Pastures + 0.6 % grain of LW, T3: Pastures + 1.2 % grain of LW, T4: concentrate ad libitum plus alfalfa hay

Carcasses from T1 had lower temperatures than the other three treatments at 24 h pm, T3 and T4 having the highest carcass temperatures (Table 5). This is consistent with results reported by Realini et al. (2004) when carcasses from pasture and concentrate diets were compared. The larger fat proportion obtained in pistola cuts from T3 and T4, could probably have an effect on post mortem chilling.

Table 5. Carcass temperature at 24 hours *post mortem*.

General mean, residual typical deviation (σ_r), coefficient of variation (CV), difference between treatments, Standard error (SE) and *p* value.

Characteristic	General mean	σ_r	CV	Contrast	Difference	SE	<i>p</i> value
Temp 24 (°C)	5.88	0.706	12.00	T1-T2	-2.3322	0.2277	<.0001
				T1-T3	-3.1924	0.2262	<.0001
				T1-T4	-3.4641	0.2796	<.0001
				T2-T3	-0.8602	0.2252	0.0003
				T2-T4	-1.1319	0.2882	0.0002
				T3-T4	-0.2717	0.2766	0.3293

T1: Pastures, T2: Pastures + 0.6 % grain of LW, T3: Pastures + 1.2 % grain of LW, T4: concentrate ad libitum plus alfalfa hay

Carcasses from T1 had higher L* fat values than carcasses from intermediate treatments and from T4 (Table 6). The same results were shown by Realini et al. (2004) when comparing grass and concentrate-fed animals.

Table 6. Subcutaneous fat colour at 24 h *post mortem*.

General mean, residual typical deviation (σ_r), coefficient of variation (CV), difference between treatments, standard error (SE) and *p* value.

Characteristic	General mean	σ_r	CV	Contrast	Difference	SE	<i>p</i> value
L*	76.35	1.997	2.61	T1 – T2	1.9263	0.6443	0.0038
				T1 – T3	4.7703	0.6399	<.0001
				T1 – T4	2.4746	0.7928	0.0026
				T2 – T3	2.8440	0.6373	<.0001
				T2 – T4	0.5484	0.8163	0.5039
				T3 – T4	-2.2956	0.7848	0.0046
a*	5.01	1.341	26.77	T1 – T2	-0.0682	0.4329	0.875
				T1 – T3	0.1563	0.4300	0.717
				T1 – T4	-2.0634	0.5327	0.000
				T2 – T3	0.2245	0.4282	0.602
				T2 – T4	-1.9952	0.5484	0.001
				T3 – T4	-2.2197	0.5273	<.0001
b*	17.20	1.538	8.94	T1 – T2	1.3143	0.4963	0.0100
				T1 – T3	1.8620	0.4930	0.0003
				T1 – T4	3.2290	0.6107	<.0001
				T2 – T3	0.5477	0.4909	0.2684
				T2 – T4	1.9147	0.6288	0.0033
				T3 – T4	1.3671	0.6045	0.0268

T1: Pastures, T2: Pastures + 0.6 % grain of LW, T3: Pastures + 1.2 % grain of LW, T4: concentrate ad libitum plus alfalfa hay

Concentrate-fed animals had higher a* fat values compared to the other three treatments, indicating more redness in subcutaneous fat (Table 6). Kerth et al. (2007) did not find differences in L* and a* fat values when comparing grass and concentrate-fed steers.

Fat b* values were higher in T1 than in the other treatments. The most relevant difference was obtained when comparing T1–T4 (Table 6). Finishing strategy, pasture characteristics, animal growth rate and animal age at slaughter seem to have an effect on fat b* value. Finishing cattle on pasture increased b* values in subcutaneous fat, indicating more yellowness compared with fat from the other treatments. Other studies have consistently shown that feedlot-finished cattle have whiter fat colour than pasture-fed ones (Realini et al., 2004). Fat colour is strongly dependent on its carotenoid content. Green and fresh pastures usually contain high quantities of carotenoids (up to 550 ppm of dry matter), whereas most of the grains contain low carotenoid concentrations (<5 ppm) (Tume and Yang, 1996). Yang et al. (2002) demonstrated that β-carotene in plasma, muscle and adipose tissues increased

the longer cattle graze on pastures. β -carotene levels increased by more than 50% in cattle finished on pastures compared with those finished on grain. Differences in the rate of fat deposition could also have contributed to differences in fat colour, due to different rates of dilution of the total carotenoid content (French et al., 2000). b^* values showed a reduction of 0.0364 units for each 1% of increase in the use of high-energy food in the diet (Fig. 2). However, Kerth et al. (2007) suggested that trimming subcutaneous fat from cuts from forage-fed cattle to 0.3 cm, reduced fat yellowness to similar levels to cuts from high-energy-fed cattle. Furthermore, carcass fabrication involves the trimming of almost all cuts nowadays. It seems that one of the most common complaints of pasture-finished beef in the United States and other countries seems not to be a problem any more, considering consumer acceptance (Kerth et al., 2007) although it could be a disadvantage in others that prefer white fat, such as Spain or Italy.

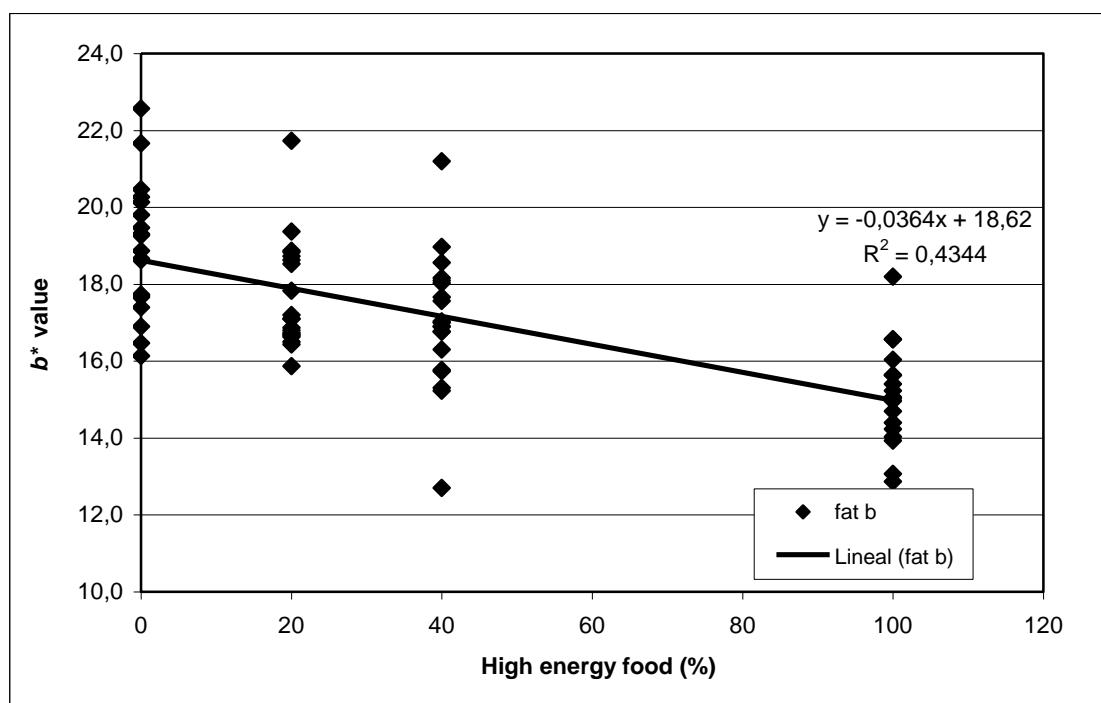


Figure 2. Fat yellowness by increasing level of energy in the diet.

T1: Pastures, T2: Pastures + 0.6 % grain of LW, T3: Pastures + 1.2 % grain of LW, T4: concentrate ad libitum plus alfalfa hay.

There was no effect of the diet on muscle colour after 7-days of aging. After 20 days, no differences were found in a^* meat values among treatments based on pastures (T1, T2 and T3), but all of them had higher a^* values than T4 (Table 7). Meat colour depends on myoglobin concentration and its chemical state, surface structure and intramuscular fat (Judge et al., 1989). Myoglobin is mainly responsible for meat colour, and a darker muscle (a^* value) could be attributed to higher myoglobin concentrations (Johansson et al., 1998).

Diet, animal age and exercise could have an affect on meat colour. High-energy diets decrease hemin pigment concentrations and consequently reduce a^* values (Lawrie, 1998). Bennett et al. (1995) obtained similar results for a^* values when comparing forage-fed animals with concentrate-fed animals. As those fed forage were older at slaughter, these authors suggested that muscle colour increase with age. In this experiment, concentrate-fed animals reached slaughter end-point earlier than other treatments, so they could have had less myoglobin concentration due to their age. Moreover, all grass-based animals could have had higher muscle myoglobin concentration, due to more pre-slaughter activity than their concentrate counterparts (Varnam and Sutherland, 1995).

Table 7. Meat colour L^* , a^* , b^* after 20 aging days.

General mean, residual typical deviation (σ_r), coefficient of variation (CV), difference between treatments, standard error (SE) and p value.

Characteristic	General mean	σ_r	CV	Contrast	Difference	SE	p value
L^*	39.77	1.619	4.069	T1-T2	-0.2007	0.5222	0.7019
				T1-T3	-1.0683	0.5255	0.0459
				T1-T4	-0.8466	0.6427	0.1920
				T2-T3	-0.8676	0.5243	0.1024
				T2-T4	-0.6459	0.6616	0.3323
				T3-T4	0.2217	0.6437	0.7316
a^*	18.92	2.637	13.94	T1-T2	-0.0339	0.8505	0.9683
				T1-T3	-0.3218	0.8563	0.7082
				T1-T4	2.2868	1.0444	0.0318
				T2-T3	-0.2878	0.8538	0.7370
				T2-T4	2.3207	1.0767	0.0345
				T3-T4	2.6086	1.0456	0.0149
b^*	11.02	1.243	11.28	T1-T2	-0.4607	0.4009	0.2543
				T1-T3	-0.6546	0.4036	0.1092
				T1-T4	0.7280	0.4922	0.1436
				T2-T3	-0.1940	0.4024	0.6313
				T2-T4	1.1886	0.5075	0.0220
				T3-T4	1.3826	0.4928	0.0065

T1: Pastures, T2: Pastures + 0.6 % grain of LW, T3: Pastures + 1.2 % grain of LW, T4: concentrate ad libitum plus alfalfa hay

No differences were found in L^* values. Intermediate treatments produced higher b^* values than T4, but these differences were not considered relevant (economical criteria) (Table 7). Realini et al. (2004) indicated that pasture-fed animals had lower L^* beef values than concentrate-fed ones, but did not find differences in a^* or b^* LD values. Several researchers did not find significant effects of different forage/concentrate ratio diets during finishing on muscle colour (Cerdeño et al., 2006; French et al., 2001). Some authors suggest that diet characteristics do not have capital relevance on meat colour, probably due to transformation processes that take place in the rumen (Albertí et al., 1992; Hedrick et al., 1983).

Shear force values were lower in T1 and T3 at 7-days of aging (Table 8). Differences were considered relevant only when comparing meat from the pasture treatment (T1) with that from the concentrate-based one (T4).

Table 8. Shear force (WBSF, Kg) after 7 and 20 aging days.

General mean, residual typical deviation (σ_r), coefficient of variation (CV), difference between treatments, standard error (SE) and *p* value.

Characteristic	General mean	σ_r	CV	Contrast	Difference	SE	<i>p</i> value
WBSF 7 days (N)	37.85	1.039	26.88	T1-T2	-0.9999	0.3437	0.0049
				T1-T3	-0.4040	0.3375	0.2354
				T1-T4	-1.3377	0.4121	0.0018
				T2-T3	0.5960	0.3344	0.0791
				T2-T4	-0.3378	0.4271	0.4316
				T3-T4	-0.9338	0.4084	0.0253
WBSF 20 days (N)	33.93	0.668	19.28	T1-T2	-0.8552	0.2152	0.0002
				T1-T3	-0.2724	0.2167	0.2128
				T1-T4	-1.0914	0.2643	<.0001
				T2-T3	0.5828	0.2160	0.0087
				T2-T4	-0.2361	0.2725	0.3892
				T3-T4	-0.8189	0.2646	0.0028

T1: Pastures, T2: Pastures + 0.6 % grain of LW, T3: Pastures + 1.2 % grain of LW, T4: concentrate ad libitum plus alfalfa hay

After 20 days of aging (Table 8), shear force followed the same pattern as with 7-days. Differences were relevant when comparing T1 with T4. This result is consistent with those obtained by Realini et al. (2004), who reported 2.8 and 3.5 kg (shear force values), for pasture and concentrate-fed animals, respectively. Also, Oltjen et al. (1971) reported that beef from steers fed on all-forage diet had higher tenderness values than beef from animals fed on all-concentrate diet.

Different external factors such as diet, pre-slaughter growth rate, animal age and the length of the finishing period, may affect beef tenderness. Finishing strategies in cattle have been extensively studied over the years with contradictory results on meat quality and carcass traits. Fishell et al. (1985) reported that cattle with high growth rate prior to slaughter had more tender meat than those with slow growth rate. Higher ADG is supposed to increase rates of protein turnover, resulting in higher concentrations of proteolytic enzymes in the carcass tissue at slaughter (Miller et al., 1987) and more soluble collagen. Fast growing groups reach market weights earlier than slow growing animals, and are therefore younger at slaughter, within a market category. Age at slaughter has been shown to be related to meat tenderness (Perry and Thompson, 2005). However, several authors did not find any decrease in shear force, when comparing steers with different pre-slaughter growing rates (Cox et al., 2006; Moloney et al., 2000). Some authors indicated that meat quality differences

between diets are minimised when forage-fed cattle are compared with concentrate-finished cattle at a common end-point such as body weight, fat thickness or degree of marbling (Crouse et al., 1984). Other authors reported that decreasing tenderness in forage-fed beef is related to the length of the finishing period (French et al., 2001). In this experiment, animals finished on pastures (216 days on treatment and with lower ADG) were more tender than the ones finished on feedlot (101 days on treatment and higher ADG).

There were no effects of dietary treatment on cooking losses at 7 and 20 days post slaughter. Similar results were found by other authors when comparing carcasses from different finishing strategies (Cerdeño et al., 2006; Kerth et al., 2007). However, Hedrick et al. (1983) reported higher cooking loss on grain-fed beef when compared with forage-fed cattle. These differences could be explained by the higher backfat registered by these authors with the higher energy diets.

3.3. Descriptive principal component analysis (PCA) for carcass, meat and fat quality traits

The first three PCs explained 71% of the total variation (31%, 24% and 16%, respectively), when performed on some carcass variables and meat quality data.

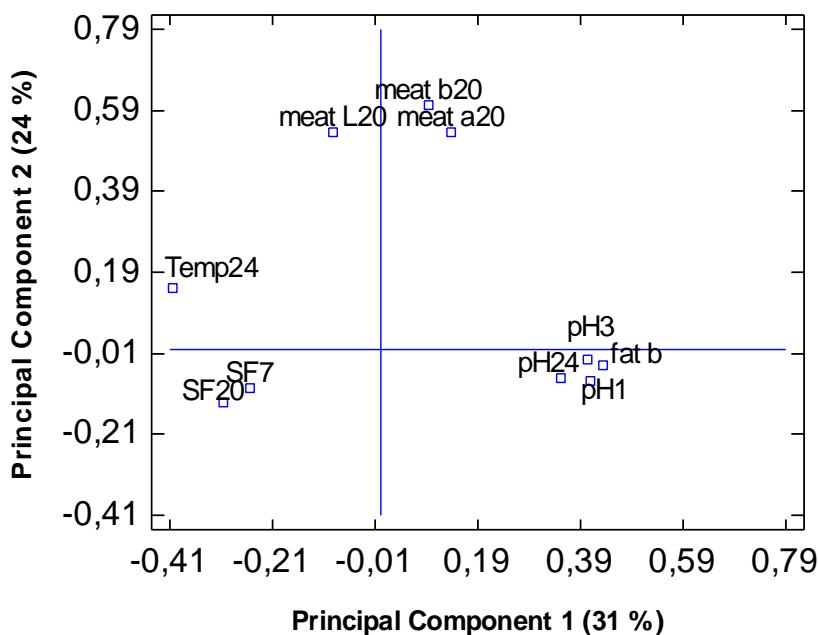


Figure 3. Similarity map in a Principal Components assay defined by PCA 1 (31 %) and PCA 2 (24%).

Abbreviations: Temp 24 – Temperature at 24 hours post mortem; pH 1, 3, 24 – pH at 1, 3 and 24 hours post mortem; meat L* 20, meat a* 20, meat b* 20 – lean colour L*, a* and b* values, at 20 aging days; fat b* – fat colour (b* value); SF 7, 14 – Shear force at 7 and 20 days of aging.

It was possible to define two groups of variables within the first PC and far from the origin, which means that they are predominant in defining this PC. One group included variables relative to the muscle-meat transformation process (pH decline) and also fat yellowness. These variables were positively correlated to each other and negatively correlated to the other group composed of shear force at 7 and 20 days, and carcass temperature at 24 h pm. The second PC (Fig. 3) was an independent source of variation, mostly defined by meat colour on one side far from the origin, and on the other side by pH values, fat yellowness and shear force at 7 and 20 days of aging.

Four different types of points were seen when projecting all the data in the first two PCs (Fig. 4), but only two feeding strategies are clearly discriminated. Pasture fed animals were on the right side of the origin and grain-fed animals on the left. Intermediate treatments were not clearly differentiated. It is clear that T1 and T4 were mainly separated by shear force and fat yellowness.

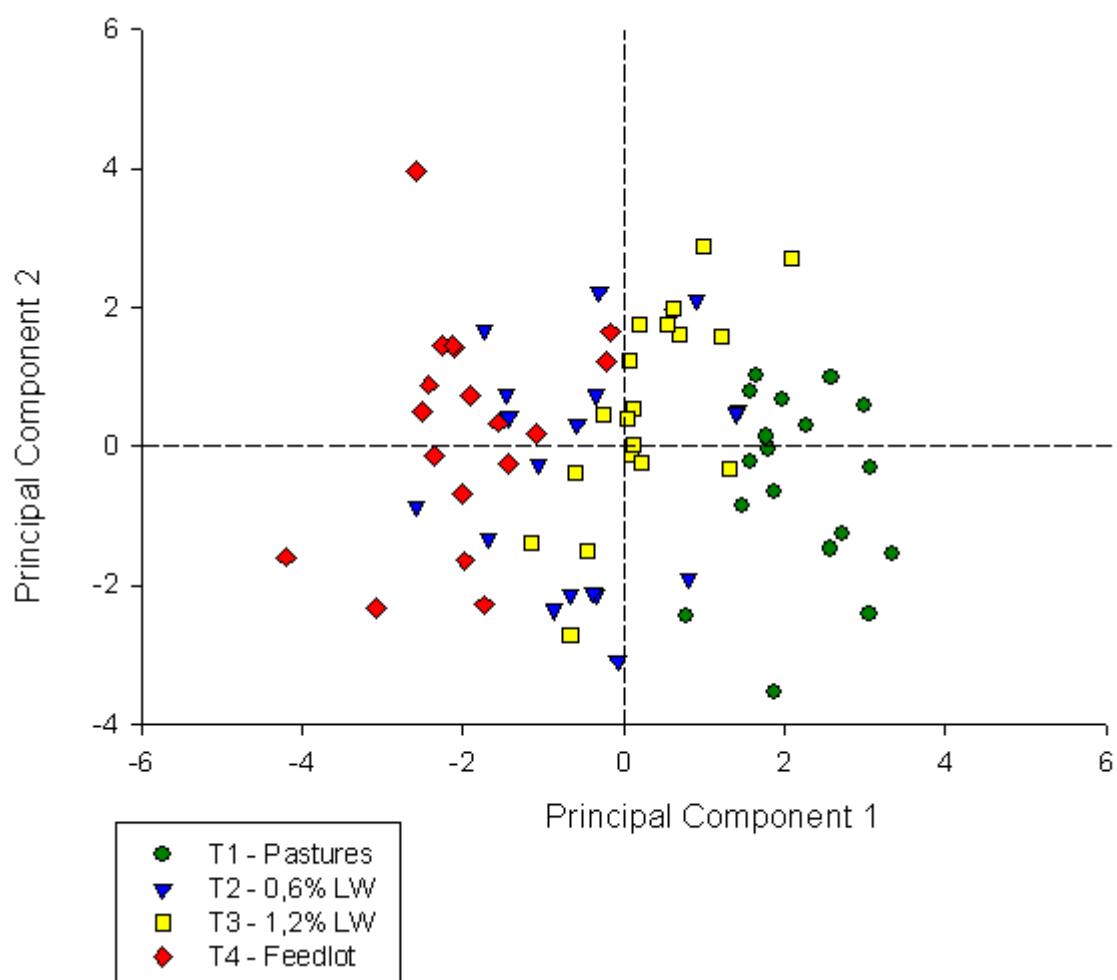


Figure 4. Projection of all the data in the space defined by the first two PCs.

4. Conclusions

Extensive production systems based mainly on pastures have been conventionally associated with some inferior meat quality attributes (tenderness, colour). However, in this study the finishing strategy did not show clear changes in meat quality. Thus, pastures finishing strategy not only did not clearly affect quality, but also increased beef tenderness. According to the experiment conditions, the use of concentrates to finish cattle is a matter of money and time, not a quality issue.

5. Implications

Feed costs are the most important factors in determining the commercial efficiency of any beef system. In certain regions, better grass utilization could be the cheapest source, especially considering fluctuations in grain prices. Commercial aspects that involve the use of concentrates and/or the extra time needed to finish animals with pasture should be taken into consideration with a view to shareholders' interests. Alternative methods for finishing cattle may be viable as long as they are profitable for producers, plant processors and they are accepted by consumers. To maximize profitability and attain different markets, potential animal growth could be achieved by an inclusion of concentrates in a forage-based diet, considering commercial issues and without any impairment of quality. Meat quality should include other aspects of increasing importance, such as fatty-acid composition (PUFA/SFA, n6/n3), oxidation rates of fat and pigments, cholesterol content, sensory quality (acceptability, intensity of odors and flavors, textural properties), animal welfare and sustainability. Some of them were analysed in this project and will be presented in others papers.

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CAPÍTULO V - ARTÍCULO 2

CAPÍTULO V - Effect of different finishing strategies on animal welfare and beef quality in Uruguay⁴

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Abstract

Eighty-four steers were randomly assigned to T1: pasture (4% of animal live weight: LW), T2: pasture (3% LW) plus concentrate (0.6% LW), T3: pasture (3% LW) plus concentrate (1.2% LW), and to an *ad libitum* concentrate treatment, T4, to study the effects on animal welfare and meat quality. Differences in average daily gain (T4>T3>T2>T1) were due to the different energetic composition of the diets and were not attributable to animal welfare problems. Animals from T4 had the best performance but also showed diet problems, a harder habituation process to the new conditions, higher values of acute phase proteins at slaughter and a higher death rate. Also, certain relevant behaviour patterns were restricted or deprived in the confined system. T1, T2 and T3 did not appear to compromise animal welfare. However, special considerations must be taken into account in the habituation process to any new diet, especially in a feedlot system. No adverse effects on meat quality were detected in T1 and tenderness was enhanced. Calmer animals had higher average daily gain and lower shear force values, so temperament appears to have a significant influence on productivity and also on meat quality.

Keywords: acute phase proteins, beef quality, fecal glucocorticoids, steers, temperament

1. Introduction

Public sensitivity to animal welfare has risen considerably in recent years and is now a strong issue in developed countries. The welfare of an individual is considered to be "its state as regards its attempts to cope with its environment" (Broom, 1986 a). Coping difficulty or failure to cope may be associated with pain or other suffering. It is generally accepted that welfare assessment requires the combination of several indicators (Broom and Johnson 1993) based on health, longevity, productive and reproductive success, disturbances to behaviour and physiology, and measures of animal preferences or motivations (Duncan and Fraser, 2005).

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Animals can be stressed by either psychological or physical factors and their reaction will depend on a complex interaction of genetics, previous experience and the quality of human handling. Stimulation of the sympatho-adrenal and hypothalamo-adrenal systems results in the production of catecholamine and corticosteroid hormones, being primarily an adaptive mechanism that allows the animal to cope with the stressor by mobilizing body reserves and regulating inflammatory response to injury (Morrow et. al., 2002). However, sustained or long-term increases in the hypothalamo-pituitary-adrenal axis (HPA) activity, are thought to be detrimental (Blecha, 2000; Broom, 1995). Circulating glucocorticoids are metabolized (conjugated) and excreted via the urine and faeces. Faecal glucocorticoid metabolite (FGM) assays reflect the average level over a time period rather than a point sample, and therefore may provide a more accurate assessment of long-term glucocorticoid levels (Harper and Austad, 2000). In ruminants, the concentration of cortisol metabolites in a faecal sample reflects cortisol production about 12 hours earlier (Mostl et al., 2002).

The acute phase proteins (APP) are a group of blood proteins whose concentration changes in animals subjected to external or internal challenges including stress, so APP analysis may be used to help monitor the health and welfare of production animals on the farm (Eckersall, 2000) or to identify the disease and health status of animals at slaughter.

Temperament may affect animal response to stress (Grandin, 1997) as well as their reaction to handling (Le Neindre et al., 1995). The use of temperament to typify stress responsiveness may prove to be valuable for models of HPA bovine function (Curley et al., 2008). Cattle with wilder temperaments exhibit lower body weight gain (Burrow, 1997; Voisinet et al., 1997a), produce tougher meat (Voisinet et al., 1997b) and yield increased amounts of bruise trim due to injuries acquired during transportation (Fordyce et al., 1988) and lairage.

Behaviour has emerged as a focal point for welfare deliberation and it could be highly related to feelings (Rollin, 2003). Positive experiences like comfort, play, exploration and contentment should be considered as criteria for welfare (Fraser, 1993).

Uruguayan meat has traditionally been produced on pastures. However, intensive systems have recently become more widespread, with a wide range of pasture and concentrate utilization in order to supply different market requirements. The objective of this experiment was therefore to evaluate the effect of the emerging systems on animal welfare and their effects on meat quality.

2. Materials and methods

2.1 Animals and diets

This study was run by the National Institute of Agricultural Research, at INIA La Estanzuela Research Station, Uruguay (Latitude 34° 20' 18" South, Longitude 57° 41' 24" West) over a period of 8 months (from September 2005 to April 2006). Eighty four Hereford steers from the same origin and backgrounded on pasture were finished on one of the following treatments (T): T1) pasture (*alfalfa-Medicago sativa*, white clover-*Trifolium repens* and fescue-*Festuca arundinacea*) offered at 4 % of live weight (LW), T2) pasture offered at 3 % of LW and concentrate (corn 0.6% of LW), T3) pasture offered at 3 % of LW and concentrate (corn 1.2% of LW), and T4) concentrate (corn, sunflower pellets and hay) *ad libitum*. Steers from T4 were located in 3 open-air plots on concrete flooring (8 square meters per animal). Animals from all treatments had *ad libitum* access to water and mineral salts.

2.2. Field determinations

Productivity measurements

Animals were weighed early in the morning without previous fasting every 14 days. Every 28 days, fat thickness and ribeye area were registered by ultrasound techniques.

Tameness and temperament

Tameness and temperament were evaluated by both non-restrained and restrained tests based on existing or adapted methodologies proposed by different authors (Burrow, 1988; Fordyce et al., 1982; Grandin and Deesing, 1998; Hemsworth et al., 1989; Le Neindre et al., 1995). The Flight zone was registered twice for each treatment (the first day and 2 days before slaughter). The test was performed by the same person who slowly walked (2 steps/second) toward the group of animals, registering the distance (meters) when half of them turned away. Animals were in subgroups of 7, so an average value was calculated for each treatment. Hair whorl position was recorded on the starting day, looking for correlations to temperament. If the center of the hair whorl was above the top of the eyes, the animal was categorized as "excitable"; "middle" if the center was located at eye level and "calm" if the center was located below the bottom of the eyes. Temperament ratings were assessed individually by different tests, once a month, after ultrasound measurements: a) Crush score (CS): the behaviour of each animal was assessed when put into a crush using a 1 (calm) to 5 (combative) scale. Each animal was held by the neck while in the squeeze chute. The rating was made after the head was clamped in the stanchion, b) Flight-time (FT): the amount of time it takes an animal to cover a fixed distance (5 meters) after it leaves the chute was recorded, and c) Exit speed (ES) from the squeeze chute was registered and was ranked as

1 = walked, 2 = trotted, and 3 if the animal ran out of the chute. A multicriterial temperament index (Tindex) was built from FT, CS and ES.

Physiological indicators

Individual jugular blood samples were collected once a month and also during bleeding in the abattoir. Blood samples were kept cool until they arrived at the laboratory when they were centrifuged at 3000 rpm for 15 minutes. The serum fractions were frozen and sent to Barcelona University (UAB), Spain, for Acute phase proteins (APP) concentration determination. APP-haptoglobin was measured in serum with a plate reader, ELISA (EMS Reader MF V2.9-0. Methodology: Bovine Haptoglobin ELISA test Kit, Life Diagnostics, Inc. Catalog number: 2410-7; Westchester, PA). To prevent haemolysis effects, Hiss et al. (2003) were followed. Results are expressed in µg/ml.

Fresh fecal samples were taken directly from the rectum of all steers before each ultrasound measurement (once a month). They were cooled for 15 minutes and immediately frozen and stored at -20°C until being sent to the University of Zaragoza, Spain, for cortisol/corticosteroids concentration determination. FGM were measured according to Morrow et al. (2002) using the commercially available I¹²⁵ radioimmunoassay kit (Rats & Mice Corticosterone kit; ICN Pharmaceuticals). Samples were analyzed in duplicate. The inter and intraassay coefficients of variation for faecal control samples were 4.3 and 6.7%, respectively. Results are expressed as nanograms of metabolite per gram of wet faeces.

Behaviour

Cattle behaviour was evaluated 4 times in all treatments by direct observation using the instantaneous scan sampling technique (Martin and Bateson, 1993) within a 15-minute sample interval. Observations were made from dawn to dusk by four trained observers, who rotated each 2 hours between treatments to minimise observer effect. At each scan, the following states/events were recorded: lying or standing (LorS), walking, grazing, ruminating, drinking water, and others (playing). Animals from each treatment were divided in three pens of 7 animals during the whole experiment (by electric fencing in pasture-based treatments and different plots in T4), for estimating pasture/concentrate ingestion and rejects. For behavioral observations, each subgroup was considered the experimental unit within each treatment.

Health Status

Any pathological event or trauma and the consequent medical treatments were observed and registered daily during the entire experimental period.

2.3 Slaughter and sampling procedures

Animals were slaughtered in a commercial abattoir licensed for export following standards procedures for Animal Welfare, when they reached an average of 500 kg of LW in each treatment and at least 6 mm of fat covering (by ultrasound technique). Growth rates differed for all treatments and consequently concentrate-fed animals (T4) were slaughtered on December 10th, 2005; followed by steers from T3 (December 27th, 2005), from T2 (January 24th, 2006) and from T1 on April 4th, 2006. Good management practices were followed during all pre-slaughter stages (transport, loading and unloading, and at the abattoir).

Carcass traits and meat quality

pH was measured at 24 hs *post mortem* (pm) in the *Longissimus thoracis* (LT) muscle between 12-13th rib using a pHmeter (Orion 210A) with gel device. After 48 hours, two steaks were vacuum packaged individually and aged for 7 and 20 days at 2-4 °C to determine tenderness (Warner Bratzler model D2000-WB). The LT steaks were placed inside polyethylene bags and cooked in a water bath until an internal temperature of 70°C was achieved, using a Barnant 115 thermometer with type E thermocouple. Six 1.27 cm diameter cores were removed from each steak parallel to the muscle fiber orientation. A single peak shear force measurement was obtained for each core using the WB and an average value was calculated for each steak.

2.4 Statistical analysis

Exploratory analyses were performed for all variables, using Statgraphics and SAS packages.

Productivity. A general linear model (PROC GLM; SAS, 2007) was used to evaluate the effect of diet and temperament on productive variables. All interactions were considered and when they were not significant they were removed from the model.

Temperament. Relationships between the initial and subsequent temperament measurements were analyzed in order to verify the consistency of each method. The three initial temperament appraisals were positively correlated with subsequent temperament assessments. Based on this exploratory analysis, a multicriterial temperament index (Tindex) was built from FT, CS and ES. A standardised ranking was created for each variable in a 1-100 scale. Tindex was created by: Tindex= $\sum W.d$, where "W" is the weight assigned to each variable estimated by the Analytic Hierarchy Process-AHP (Saaty, 1980); and "d" is each individual record, standardised (within each variable). Considering that FT is an objective test, it was assigned a relatively higher ranking in the index. The higher Tindex, the calmer the animal. A mixed model with repeated measures was used to evaluate treatment effect on

Tindex with time (4 consecutive dates) with the animal as a random effect inside each treatment. A regression analysis was performed to study the effect of temperament on ADG.

Physiology. Due to absence of normality, non-parametric tests (Mood's and Kruskal Wallis median test; PROC NPAR1WAY; SAS, 2007) were performed to analyse treatment effect on APP values and APP stress response due to the slaughter procedure. The same tests were applied to analyse treatment and time effect on FGM. Regression models were used to analyse the relationship between physiological indicators and ADG.

Behaviour. A classification tree analysis was performed (SPSS 16 package, 2007) to evaluate treatment effect on behaviour frequencies. A logistic regression model was used to analyze daytime patterns of each observed behaviour. Based on grazing ethology in range cattle, a polynomic 4th degree equation was fitted and differences between treatments were analysed. The logit model used was:

$$\text{Logit } P_{ijk} = \beta_0 + \beta_1 DT_i + \beta_2 DT_i^2 + \beta_3 DT_i^3 + \beta_4 DT_i^4 + T_j + \varepsilon_{ijk}$$

where Logit $P_{ij} = \log(p/1-p) = \log(\text{positive response}/\text{absence of positive response})$ on each activity

DT_i = linear coefficient for daytime

DT_i^2 = quadratic coefficient for daytime (2nd order)

DT_i^3 = cubic coefficient for daytime (3rd order)

DT_i^4 = fourth order coefficient for daytime (4th order)

T_j = treatment effect

ε_{ijk} = experimental error

Carcass and meat quality. Analysis of variance were carried out to study the effects of different factors and variables on tenderness, using initial and final live weight as covariates (PROC GLM; SAS, 2007). Regression analysis were also carried out to evaluate the effect of different variables on tenderness. Several correlation analyses were performed on data relating to production, physiology and temperament. Means were compared by the LSMEANS procedure (SAS, 2007).

3. Results and discussion

3.1. Field determinations

Productive variables

Concentrate-fed animals (T4) had the highest average daily gain (ADG) which increased with the level of energy in the diet (Table 1). In general, when pastures and grains or

concentrates are offered *ad libitum*, ADG is higher in concentrate-fed cattle, relative to grass-fed animals (French et al., 2000; French et al., 2001). In our experiment, the use of grain even when not *ad libitum* (T2 and T3) increased ADG and decreased days to slaughtering. These differences were due to the total dry matter or digestive dry matter consumption, associated to different concentrate/energy proportion of the diet and probably to a lower energy expenditure for maintenance in T4. Fat thickness and ribeye area were also higher as long as supplementation increased (Table 1). Remaining pasture after grazing (more than 1.100 kg/dry matter) indicated that pasture consumption was not restricted in T1, T2 and T3 (Hodgson, 1968). Productivity is not enough to ascertain an adequate animal welfare status (Veissier et al., 1999) nor to establish differences between production systems, but is relevant to welfare because it testifies that at least certain animal needs are being met. In T4, animals gained weight in part because they were practically immobile, but they could have suffered because of the inability to move or to perform certain behaviours (see behaviour and physiology discussion).

Table 1. Average daily gain, ribeye area gain and fat thickness gain by treatment.
Least square means \pm standard error.

	Treatments			
	T1	T 2	T3	T4
Average daily gain (kg/an)	0.52 ^d \pm 0.05	0.94 ^c \pm 0.05	1.12 ^b \pm 0.05	1.56 ^a \pm 0.05
Ribeye area gain (cm ² /day)	0.05 ^b \pm 0.01	0.08 ^b \pm 0.01	0.08 ^b \pm 0.01	0.17 ^a \pm 0.01
Fat thickness gain (mm/day)	0.03 ^c \pm 0.00	0.04 ^b \pm 0.00	0.06 ^a \pm 0.00	0.07 ^a \pm 0.00

^{a,b,c,d} Means within the same line with different letter differ with P<0.05

Temperament

In this experiment, hair whorl position was related neither to Tindex nor to productive variables. These results are not consistent with reports from Grandin et al. (1996) who indicated that cattle with a round hair whorl located above the eyes became significantly more agitated while they were restrained in a squeeze chute (crush) compared to cattle with a hair whorl located either between or below the eyes. With results similar to ours, Randle (1998) reported that cattle response to humans in general was not significantly associated with hair whorl position.

Tindex had a significant effect on liveweight gains (ADG) within each treatment (Figure 1).

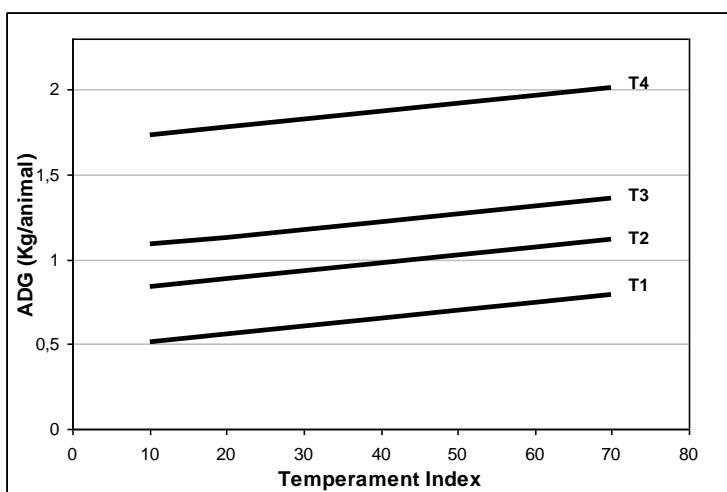


Figure 1. Average daily gains according to Tindex.
Trendlines per treatment, estimated by regression analysis ($R^2=0.85$).

Reduced growth is considered to be the consequence of a series of acute or chronic responses to human presence (Barnett, et al., 1983; Hemsworth et al., 1996). Voisin et al. (1997 a) also reported a significant effect of temperament ranking on cattle. According to their results, *Bos Taurus* steers with the calmest temperament had 0.19 kg/d greater ($P<0.05$) average daily gain than steers with the most excitable temperaments.

Figure 2 shows initial and last Tindex comparison within each treatment. It was observed that animals from intermediate treatments and from T4 became calmer as the experiment progressed. All animals were used to handling and penning because of frequent weighting and a strict protocol of good management practices was followed during the whole process, but, steers from T1 had less daily contact with humans. Animals that are used to frequent handling and close contact with people are usually less stressed by restraint and handling (Grandin, 1997).

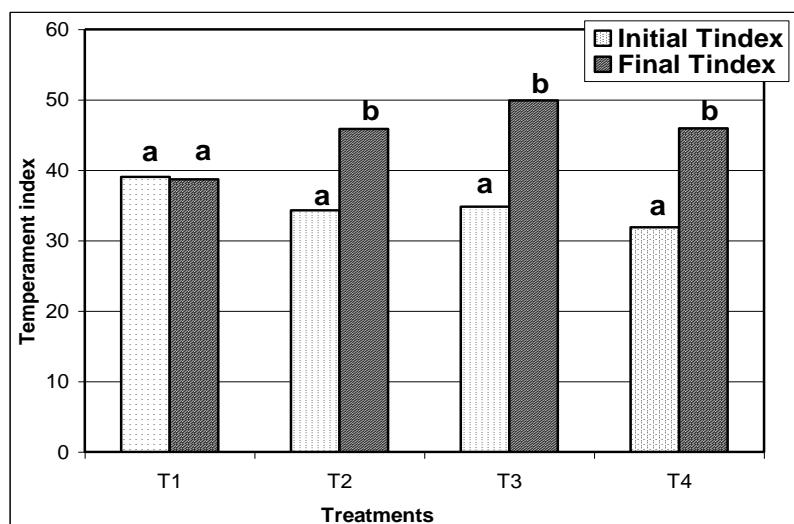


Figure 2. Least Square Means for Initial and Final Tindex within treatments.
a, b Bars within the same treatment with different letter, differ $P<0.05$

According to these results, animal temperament and human handling are key factors for minimising stress.

The Flight zone (FZ) is the animal's personal space and its size depends on the tameness of livestock. When someone enters its space, the animal will move away. Feedlot steers showed the lowest FZ at the end of the experiment (Figure 3). Le Neindre (1996) also reported that pasturing cattle had lower docility than indoor-raised animals. However, it was clear that all animals in this study were tame and were habituated to human proximity (higher final FZ was 2.5 meters in T3).

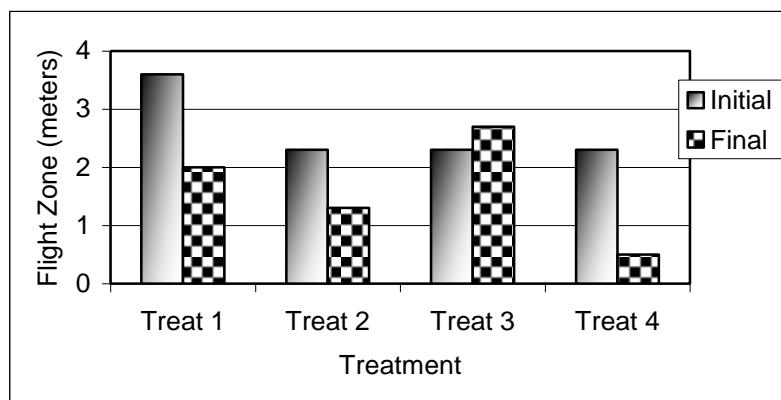


Figure 3. Mean values of Initial and final Flight Zone for each treatment.

Similar results were found by Uetake (2002) who reported that the FZ of cows was gradually reduced throughout the experimental period. It is more than likely that the good management practices followed in our experiment, had an effect on FZ values. These results suggest that the stockperson and handling procedures could be even more relevant than the rearing system in determining the flight zone.

Physiology

Bovine Haptoglobin (APP) and fecal cortisol (FGM)

APP concentration did not differ between finishing strategies within each date ($p<0.05$) but was significantly affected by the time period. Results from the Kruskal Wallis and Mood median tests showed that treatment 2 and treatment 4 had a significant rise in PFA concentration with respect to basal values during the second month of the experiment (Figure 4). These protein concentrations could change in animals subjected to external or internal challenges such as infection, inflammation, surgical trauma and also stress (Eckersall, 2000). Animals suffering illness were not considered in this analysis, but subclinical states of disease or inflammation processes not detected by us, could have contributed to these results. Anyway, APP was merely interpreted as a stress response

probably due to environmental conditions (confinement, diet, weather). Thus, even in the absence of morbidity or detected disease, a stress response as measured by APP concentrations was observed in both treatments at that date. Similar results were found by Arthington et al. (2005) when comparing stress in early and normal weaned calves by APP concentration. Although any calf became sick, these authors reported differences in APP concentration between treatments attributable to a stress response and also reported an inhibition of animal growth. Significant evidence exists that APP can directly inhibit animal growth (Johnson, 1997). However, in this experiment, APP stress response was not enough to induce variations in live weight gains ($p<0.05$). Although the mechanism of APP induction in response to stress has yet to be elucidated, activation of the hypothalamic–pituitary–adrenal (HPA) axis by stress signals may be a trigger of systemic or local (intra-pituitary) cytokine production, thereby augmenting hepatic APP synthesis and release into the bloodstream (Murata et al., 2004).

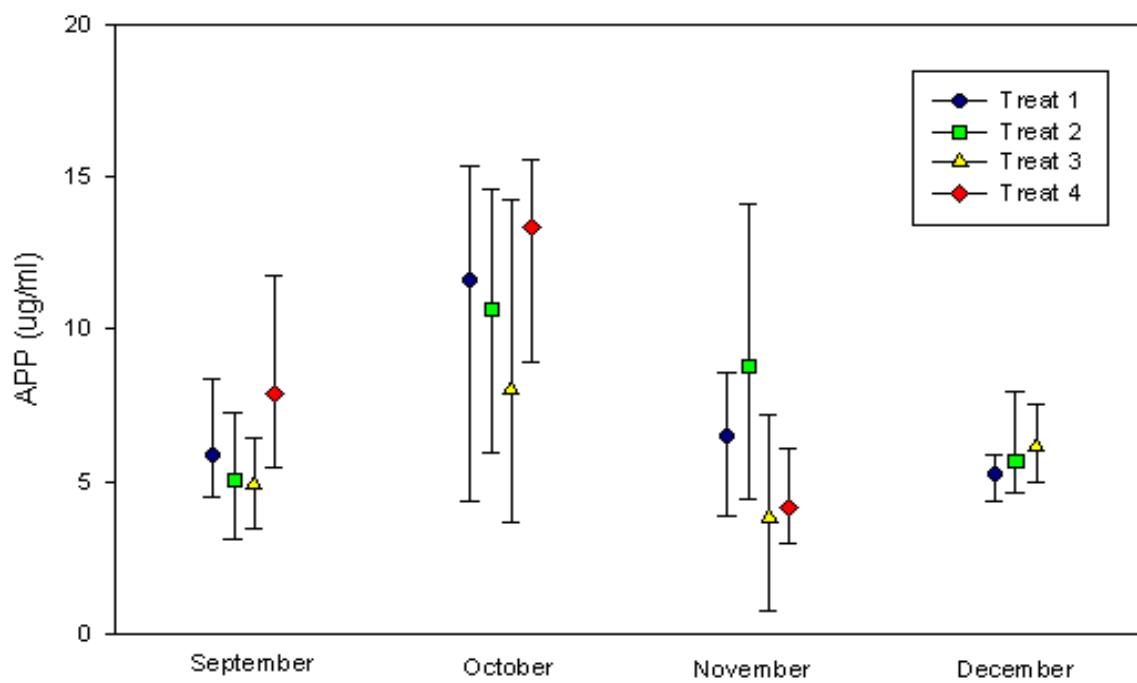


Figure 4. APP concentration in the different treatments throughout the experiment. Lines represent the median, 25th and 75th percentile values.

FGM concentration showed no differences between the finishing strategies at each date, but also was significantly affected by time ($p<0.05$). Results from the Kruskal Wallis and Mood median tests showed that all treatments had a significant decrease in FGM values during the second month of the experiment (Figure 5).

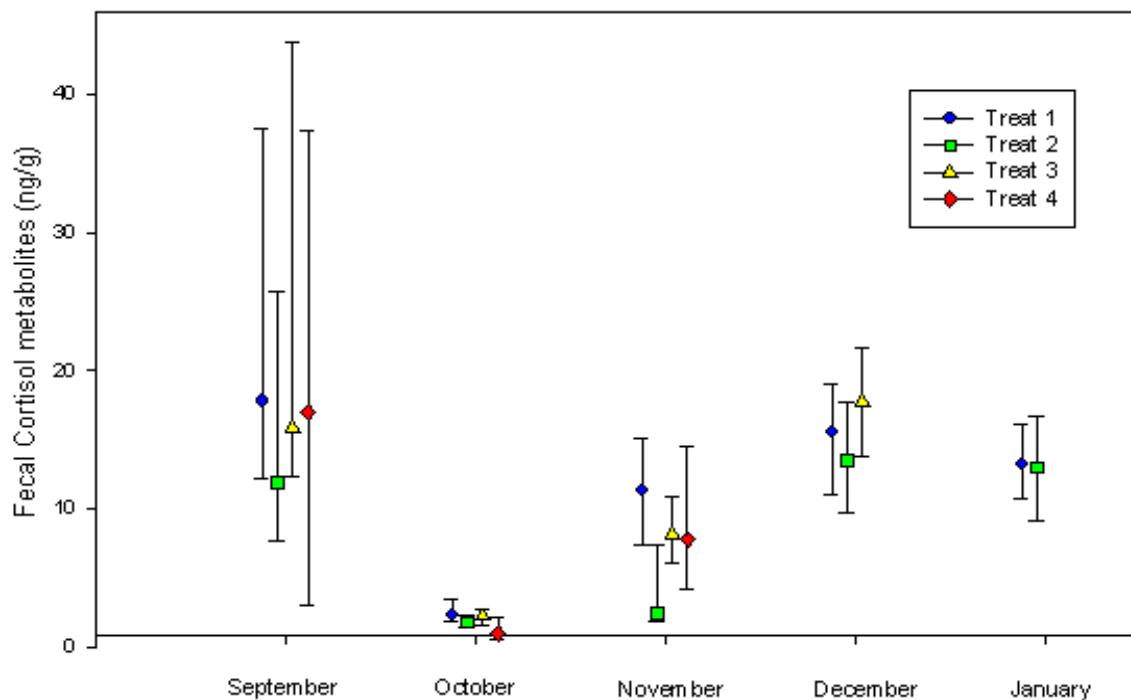


Figure 5. Fecal cortisol concentration in the different treatments throughout the experiment. Lines represent the median, 25th and 75th percentile values.

Animals from all treatments were probably suffering from long-term stress and were trying to cope with a potent stressor, which may have suppressed adrenocortical responses. The day previous to the FGM determination, a heavy rainfall was registered (40 mm; GRAS, 2008). Considering that this winter had been particularly dry and combined with a high wind (113 km/h), the precipitation could have contributed to the long-term stress. HPA activation in response to an aversive event depends on the control the animal can exercise on this event (Anderson et al., 1996). We therefore considered that the activity of the system was probably changed due to a chronic stress state which reduced the sensitivity of the HPA axis. Under these circumstances, each level of the axis is subjected to opposing influences (stimulation and also feedback through corticosteroid hormones). This resetting of the HPA axis at a different level of activity was probably the state of resistance (Selye, 1956). Similar results were found by Fisher et al. (1997) when subjecting finishing heifers to different space allowances. An increase in glucocorticoid secretion does not automatically equate to a state of distress (Moberg, 2000; Romero, 2004), but the chronic activation of the HPA axis observed in this experiment suggested a high level of stress and probably involved suffering. Animals from all treatments were able to recover normal activity of the HPA axis and returned to basal values on the following date ($p<0.05$; Figure 6). It seems that adaptation to environmental challenges at the level of the pituitary-adrenocortical system, are flexible and dynamic over time. They may involve sensitization and desensitization, for example due to reversible changes in the density and sensitivity of ACTH and cortisol receptors (Mormède et

al., 2007). Also, APP concentration returned to basal values in the following period. The stress response was considered more relevant in the feed lot system and in T2 (considering the simultaneous rise in APP). It is worth noting that in the confined system animals were space-restricted and were forced to be in permanent social contact and, which is probably more important, they were not able to perform certain relevant behaviours (see behaviour discussion). Moreover, they were probably under a subclinical acidosis state because of the high level of concentrate in the diet. All these conditions could have made the habituation process more difficult and consequently would have induced a higher stress response. However, the stress response did not result in a significant biological cost that shifted energy away from normal processes (Moberg, 2000). There was no effect of APP on ADG in any treatment, either during this period or throughout the whole experiment. In this study, temperament was related neither to the APP nor to the FGM stress response ($p<0.05$).

The preslaughter stress response was also studied through APP concentration in blood. In T1, T2 and T4, APP values at slaughter (debleeding) were higher ($P<0.05$) than pre loading determinations (Table 2). In T3, differences were not significant but a high variability was observed in APP at slaughter (Figure 6).

Table 2. APP serum concentration (ug/ml) in the last field determination and at slaughter, within each treatment. Medians and significance.

Treatments	Pre loading (ug/ml)	Slaughter APP (ug/ml)	Significance
1	10.6	14.02	*
2	5.4	12.2	**
3	5.1	5.4	ns
4	4.2	21.5	**

$p<0.05$, ** $p<0.01$, ns: not significant

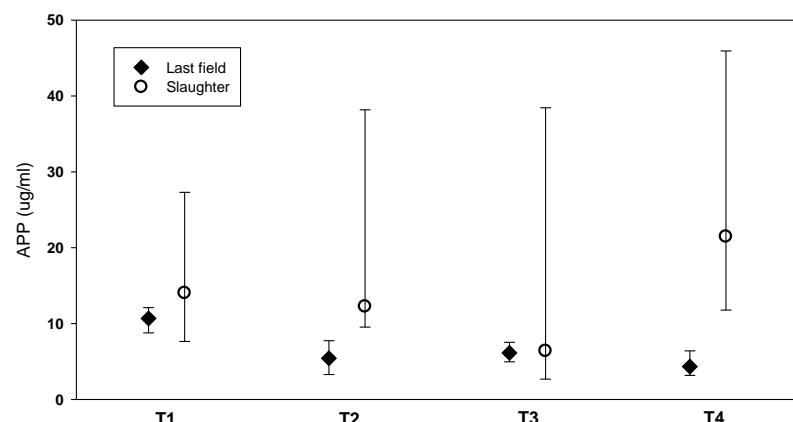


Figure 6. Last field and slaughter APP serum concentration within each treatment. Lines represent the median, 25th and 75th percentile values.

Health status

Health status in T1, T2 and T3 was satisfactory and no specific medical treatments were required throughout the experiment. Although preventive measures were applied and health monitoring was permanent, mortality was relevant in T4 because of dietary diseases, with no deaths registered in the other treatments. Health status is an important welfare indicator in farm animals and regardless of the rearing system, must be a fundamental target for animal's quality of life (Gottardo, 2004). Mortality is also a clear measure of poor welfare, not only because animals that die have obviously failed to cope, but also because high losses in a given environment show that even those individuals that do not die may have serious difficulties in coping (Manteca and Velarde, 2007). T1, T2 and T3 did not reveal differences in terms of health and mortality rate.

We previously saw that animals from the feedlot system had the highest ADG and final live weights. From a different perspective we can now say that these animals suffered higher stress throughout the experiment and at slaughter, their health was compromised and mortality was higher in this treatment. This reinforces the theory that productivity is not enough to assess an animal's welfare status. On the contrary, as in our experiment and in agreement with Rollin (2003), profitable production systems may be the cause of certain production problems and diseases.

Behaviour

Based on behaviour frequencies, all treatments were discriminated when using a classification tree multivariate procedure (SPSS v.16, 2007) (Figure 7).

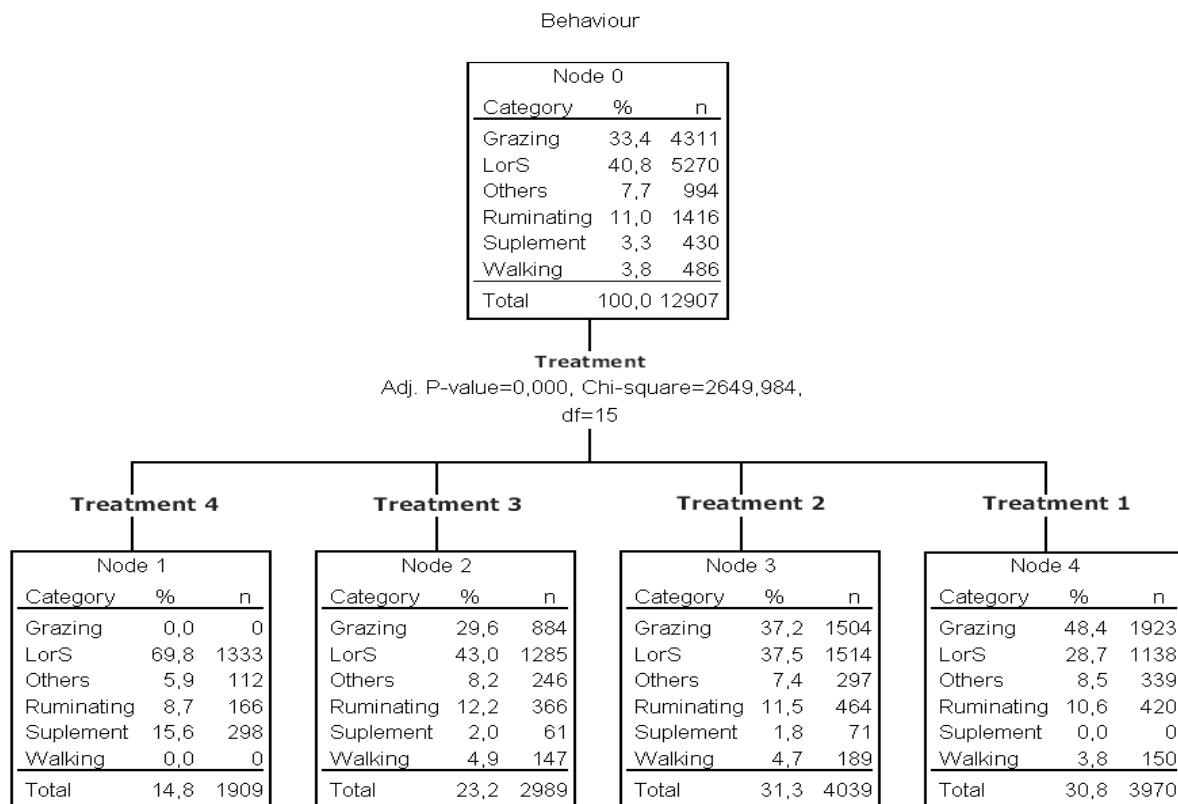


Figure 7. Treatment classification based on behaviour frequency distribution.

Table 3. 1st, 2nd, 3rd and 4th coefficients of significance for daily patterns of behaviour, and difference between treatments.

	<i>Intercept</i>	<i>Linear</i>	<i>2nd order</i>	<i>3rd order</i>	<i>4th order</i>	<i>Difference between treatments</i>
Grazing	**	**	**	**	**	T1>T2>T3
Ruminating	**	**	**	**	**	(T1=T2=T3)>T4
Supplement	**	**	**	**	**	(T2=T3)<T4
Lying/Standing	**	**	**	ns	**	T1<T2<T3<T4

* p< 0.05, **p< 0.01, ns: non significant

Cattle normally have daily alternate periods of grazing, ruminating and resting (Galli et al., 1996).

Grazing. Animals from T1 spent more time of the day grazing when compared to T2 and T3 (Table 3). Similar results were found by Wagnon (1963) who showed that daily feeding of supplements disrupted normal grazing activity and reduced daily grazing time in range cows. Grazing time in cattle is between 4 and 14 hours per day, with most observations between 7 and 11 hours (Galli et al., 1996). According to Poppi et al. (1987), if grazing time is more than 12-13 hours per day, ingestive behaviour components could be affected. Hodgson (1990) considers that grazing time higher than 9 hours might indicate some kind of pasture

restriction. In our experiment, grazing time was 6 hours in T1, 4.5 hours in T2 and 3.6 hours in T3, but these differences in time spent grazing were not considered relevant to animal welfare, especially considering the high quality and availability of the pasture. In addition, we did not register night grazing behaviour, which was probably important. Although grazing behaviour is affected by various environmental conditions (Adams and Reynolds, 1983), most grazing behaviour studies show similarity in daily grazing patterns, with the major grazing period occurring early in the morning and another later in the afternoon, with intermittent grazing occurring throughout other periods of the day and night (baseline ethogram) (Dwyer, 1961). In our experiment, periodical grazing patterns were not altered due to the supplementation effect (Figure 8). One criterion of good animal welfare is the expression of a time budget similar to that expressed by free ranging conspecifics (Young et al., 1994). Deprivation of grazing in T4 could have affected animal welfare, considering the physiological consequences of this deprivation. However, it is necessary to investigate the motivational systems underlying each separate behaviour to reach firm conclusions about the influence of behavioural restriction on animal welfare (Petherick and Rushen, 2005).

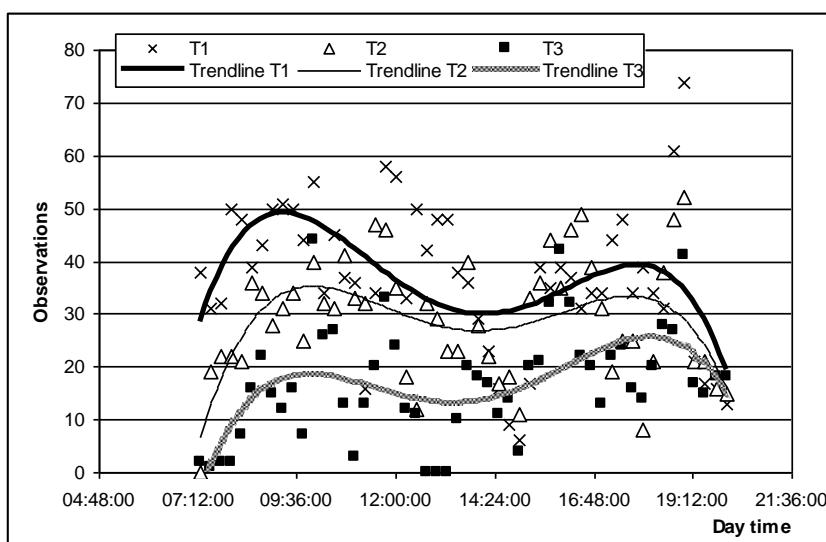


Figure 8. Daytime grazing frequency and distribution, by treatment.

Ruminating. Time spent ruminating was similar for steers in pasture-based treatments (Table 3). Similar results were found by Adams (1985) reporting no differences in ruminating time between pasture-based cows, both supplemented and non supplemented. As ruminating time may change due to cell wall material consumed (associated to pasture ingestion), these results suggested that the supplement did not substitute pasture ingestion. Time spent ruminating was lower in T4 than in other treatments (Table 3). Although hay was *ad libitum* in T4, pasture-based treatments ingested more cell wall material and consequently spent more time ruminating. Ruminating time and number of ruminating bouts are likely to affect overall

production, because of buffering the rumen with copious amounts of saliva, which enhances the rumen environment (Schutz and Pajor, 2001). In this experiment, the reduction in time ruminating did not compromise overall production in T4, but several individual problems emerged, probably due to this factor among others (dietary diseases and death). Periodic patterns of rumination are shown in Figure 9. Ruminating time could not be compared in absolute terms, because animals were not observed during the night, when they are thought to perform the highest frequency of this behaviour (Hodgson, 1990; Rovira, 1996).

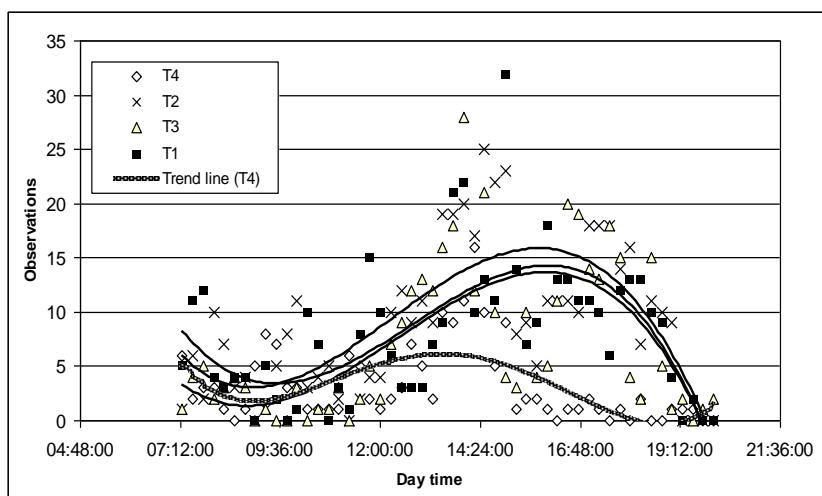


Figure 9. Day time ruminating frequency and distribution, by treatment.

Standing and lying. Animals from T3 spent more time standing and lying with intermediate values for T2 and lower for T1. Animals from T4 spent more time than pasture-based treatments standing and lying. However, it would be a subjective criterion to categorize these behaviours as “resting” in T4. Confined animals had fewer choices available and several behaviour patterns were suppressed. Animals from pasture-based treatments were able to perform several normal behaviours, but animals from T4 could not. The performance of a behaviour associated with feeding can directly influence the digestive processes even if the consumption of food and nutrient intake is not altered (de Pasillé et al., 1993). Regarding exploration, it is also reported that cattle give a very high priority to this behaviour (Kilgour, 1975) and it may be included in the “behavioural needs” category. In addition to being deprived of grazing and exploring, animals from T4 could not satisfy their individual spatial needs, they could not establish spatial distribution according to social structure, they had a more uncomfortable lying surface (no bedding area), they could not exercise, rest or roam; they suffered more from fear when threatened and were unable to escape or to adequately face situations like bad weather conditions. All these factors would have contributed to boredom, frustration, depression or despair from an unrewarding environment, and, consequently, suffering. This stressful situation in T4, may have induced physiological

consequences in order to cope with the environment, as the physiological stress response previously discussed.

Walking. Walking frequencies were not different in T3, T2 and T1 and were obviously all higher than T4 ($p<0.05$, Figure 7). Regarding Supplementing, animals from T4 spent more time eating concentrate than T3 and T2 as expected, and the former did not differ.

3.2. Carcass and meat quality

pH values at 24 hours pm were higher in carcasses from T1 than in those from T2 and T4 (Table 5). These results are consistent with several author reports, sustaining that pH decline depends on muscle glycogen concentration. Daly et al. (2002) showed that higher initial muscle glycogen concentration resulted in a faster rate of pH decline. According to Immonen et al. (2000) glycogen levels in muscle increase with metabolisable energy intake. These authors reported that the effect of a high energy diet on the muscle content was reflected all the way to ultimate pH values and residual glycogen concentrations. Muir et al. (1998) suggested that animals from pasture diets are more susceptible to pre-slaughter stress, so they could have less glycogen content in muscle and consequently higher final and intermediate pH values than grain-fed steers. In spite of the differences in initial and final pH values in our experiment, all were within the normal range (Table 4) and without incidence of dark cutting beef. Moreover, shear force values (WBSF) with 7 and 20 aging days, were higher in concentrate-fed beef than in meat from forage-fed cattle (Table 4). This result is consistent with those obtained by Realini et al. (2004) who reported 2.8 and 3.5 kg (WBSF) for pasture and concentrate-fed animals, respectively. Table 4 shows that WBSF decreased in all treatments with aging time.

Table 4. pH at 24 hs pm and WBSF (KgF) after 7 and 20 aging days by treatment.
Least square means \pm standard error.

	<i>Treatments</i>			
	1	2	3	4
pH 24 hs	5.70 ^a \pm 0.03	5.54 ^b \pm 0.03	5.73 ^a \pm 0.03	5.47 ^b \pm 0.03
WBSF 7 days	3.17 ^c \pm 0.25	4.17 ^{ab} \pm 0.25	3.57 ^{bc} \pm 0.24	4.51 ^a \pm 0.30
WBSF 20 days	2.87 ^b \pm 0.16	3.78 ^a \pm 0.16	3.26 ^b \pm 0.16	3.98 ^a \pm 0.19

Values with different letter in the same line differ $p<0.05$

WBSF: Warner Bratzler shear force

Many authors reported that beef from high energy diets was more tender because of higher animal ADG (Miller et al., 1987), leading to a shorter finishing period (French et al., 2001) and consequently the slaughter point was reached earlier with younger animals (Perry and

Thompson, 2005). However, other authors indicate that differences in meat quality are minimised when forage and concentrate-fed cattle are compared at a common end point (Crouse et al., 1984). In this study, older animals with lower ADG and a longer finishing period provided the tenderest beef. This is probably due to certain effects in the pre and *post mortem* phase (such as proteolysis related to pH and temperature decline and to pre slaughter stress) which could be investigated in future studies.

Temperament had a significant effect on WBSF after 20 aging days ($p<0.05$). Calmer animals had lower WBSF values in all treatments. The interaction between treatment and Tindex was not significant, so that, slopes were parallel implying that WBSF decreased at the same rate in all systems as Tindex increased (Figure 10).

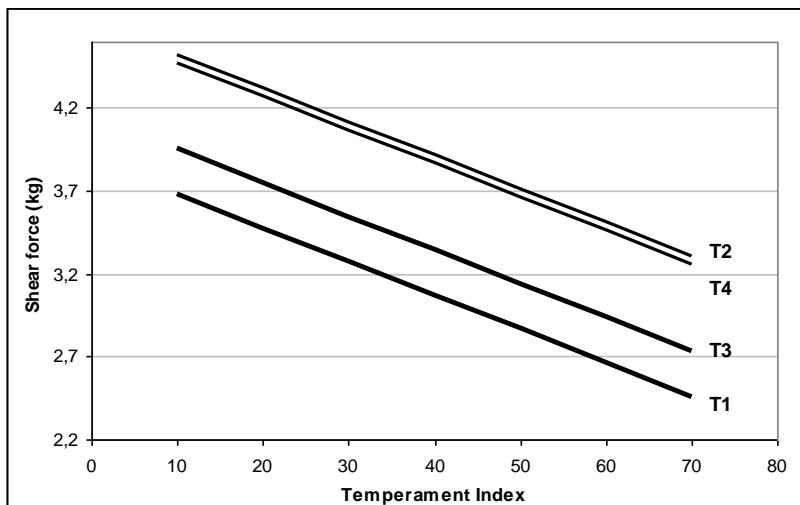


Figure 10. Shear force (Kg) with 20 aging days according to Tindex.
Trendlines per treatment, estimated by regression analysis ($R^2=0.30$).

Steers with the most excitable temperament may be most susceptible to stress generated by routine handling practices, such as loading and unloading, transport and the new environment in the abattoir (Lensink et al., 2000). This stress is likely to reduce muscle glycogen level *in vivo* (Warris, 1990) because of energy expenditure due to physical exercise or psychological stress, which may in turn increase ultimate pH of muscles (Gregory and Grandin, 1998). There were no excitable animals in this experiment and pH values were within normality, but as reaction to stress varies according to individuals, it is conceivable that additional stressors near the time of slaughter such as handling, sorting, transporting and (or) novel environments would have provided sufficient stress levels in moderately stress susceptible animals (Voisinet et al., 1997 b). Considering its commercial importance and consumer satisfaction according to WBSF (Olson, 2002), tenderness was divided into 3 categories in order to establish relationships with temperament (Table 5).

Table 5. Temperament index according to WBSF (Kg) by category.
Least square means.

	SF < 3 (100% CS)	3 > SF < 4 (99% CS)	SF > 4 (86% CS)
7 aging days	45 ^a	36 ^{ab}	26 ^b
20 aging days	40 ^a	35 ^{ab}	22 ^b

^{a,b} Means within the same line with different letter differ with P<0.05
WBSF: Warner Bratzler shear force, CS: consumer satisfaction.

With 7 aging days, beef with shear force values less than 3 (which means 100 % consumer acceptance) came from calmer animals. Beef with WBSF values from 3 to 4, came from animals with an intermediate Tindex, and the least tender beef came from the more excitable animals (Table 5). This effect was still relevant with 20 aging days. According to Voisin et al. (1997 b) a possible hypothesis for temperament effects on tenderness may involve adrenaline action on β -adrenoceptors. Adrenaline binding at β -adrenergic receptors on muscle cell membranes mediate glycogen metabolism. β -adrenoceptor agonists have been implicated in increasing shear force values (Koohmaraie et al., 1991) and β -adrenoceptor density is mildly related to darkening of muscle color (Hoey et al., 1995). From this it is possible to postulate that a relationship between β -adrenoceptor density or affinity, and temperament could possibly explain a portion of the effects on meat quality (Voisin et al., 1997 b). The correlation coefficient between Tindex and pH at 24-hours *post mortem*, partially confirms this hypothesis ($r= -0.53$; p value= 0.01). In addition, the altered metabolism associated with greater stress responsiveness in the more stressed cattle may have created conditions that were less favorable to calpain-mediated proteolysis (King et al., 2006). A high rate of protein turnover in the muscle at the time of slaughter is important to improve tenderisation, and provided pHu is low, such animals will become acceptably tender.

4. Conclusions

Productivity increased with the level of energy in the diet. In the feed lot system a longer habituation process was evident through physiological indicators and behaviour was restricted throughout the experiment. Several animals showed diet disorders in the confined system and an important mortality rate was registered in that treatment. Rainfall appeared to be a potent stressor for cattle, with no incidence on production indicators. Temperament had a significant effect on live weight gains within each treatment and frequent and proper handling improved animal reactions to regular hanling procedures. All treatments showed a considerable APP stress response at slaughter. Regardless of the feeding strategy,

temperament appears to be an important factor considering its influence on productivity and meat tenderness. Stressors that lower muscle glycogen pre-slaughter can have a significant effect on meat shear force and animals with a more excitable temperament were clearly the most susceptible. Meat tenderness was enhanced in the pasture treatment.

5. Implications

Intensification up to certain levels and without deprivation of certain behaviours should provide benefits without compromising animal welfare. However, strict preventive measures should be applied in order to avoid dietary problems, diseases and animal deaths. Feed-lot systems even when in the open air and with at least 8 m² per animal, would compromise animal welfare. Future studies should evaluate pathological and aggressive behaviour as indicators of discomfort, boredom or frustration and motivational systems underlying each separate behaviour, to reach firm conclusions about the influence of behavioural restriction on animal welfare. Human handling should be considered of paramount importance in animal welfare and meat quality, and special considerations should be taken when working with excitable animals. Immediate preslaughter stress seems to be very important and specific studies should be undertaken with a view to minimising it. Research should also be conducted regarding differences in the tenderisation biochemistry process associated with feeding strategies, temperament and pre-slaughter stress (proteolysis, glycolysis and ATP turnover).

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CAPÍTULO VI - ARTÍCULO 3

CAPÍTULO VI - Finishing diet, lairage time and temperament effects on carcass and meat quality traits in Uruguayan steers⁵

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Abstract

Sixty Hereford (H) and Braford (B) steers were assigned to two diets: D1) native pasture plus corn grain (1 % of live weight) (H n=15, B n=15); and D2) high quality pasture (H n=15, B n=15) for finishing purposes. Temperament was individually assessed during the experiment. Animals were slaughtered the same day in two groups (50 % of animals from D1 and 50 % from D2 in each group) subjected to 15 and 3 hours in lairage pens, respectively. Average daily gain (ADG) and temperament did not differ between diets. Calmer steers had higher ADG within both breeds and also showed lower meat shear force values. Animals from D1 had higher hot carcass weight (HCW), pistola cut weight (PCW) and valuable cuts weight than D2, with no differences in meat yield. There were no differences in muscle percentage between diets, but pistola cut (PC) from D1 had higher fat percentage and higher fat thickness than those from D2. Fat colour was not affected by diet, breed or lairage time. Muscle colour was affected neither by diet nor by breed, but steers from the long lairage group had a better rate of pH decline, which was linked to better meat colour (higher *a) and more tender meat. It seems that more than 3 hours pre-slaughter time is necessary to allow rest and recovery in cattle. Temperament appears to be an important factor regarding its effect on productivity and on meat quality. Braford animals were more excitable than Hereford, had higher HCW, PCW, muscle percentage in the PC, valuable cuts weight and meat yield, but meat was less tender in that breed ($p<0.05$).

Key words: steers, temperament, lairage time, diet, meat quality

1. Introduction

Uruguayan meat production systems are mainly based on pasture feeding but the use of high quality pastures and supplement for finishing purposes is becoming common nowadays, leading to cattle with different carcass and meat quality attributes that need to be studied. In many European countries it is common to slaughter animals on the day of arrival, whereas in others like Uruguay, in accordance with some meat safety regulations, animals are more typically slaughtered the day after arrival. Several authors sustain that lairage time potentially

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allows cattle to replenish muscle glycogen concentrations, reduce dehydration of body tissues and carcass weight loss and to rest and recover from some of the effects of transport. Other authors think that the lairage environment itself may inhibit the ability of cattle to rest or recover from the effects of feed and water restriction (Jarvis et al., 1996) and long lairage is associated with a drop in carcass and meat quality (Gallo et al., 2003).

The magnitude and quality of the stress response will be greatly affected by individual differences (Moberg, 1985). Animals with most excitable temperaments may be most susceptible to stress generated by routine handling practices and with the consequent negative effects on productivity (Voisinet et al., 1997 a). Also, as a consequence of reduced animal reaction to handling, energy expenditure during transport and lairage will be reduced. It is expected that under commercial conditions, energy saving as a consequence of reduced animal reaction to handling could minimize the risk of lowered meat quality (Lensink et al., 2000). Excitable cattle have been reported to have increased incidence of dark cutting lean and increased Warner–Bratzler shear force values than their calmer pen mates (Falkenberg et al., 2005; Voisinet et al., 1997 b).

Variability in results indicates that further research is required in order to establish a proper waiting time before slaughter, including the welfare perspective. The objective of this experiment was to evaluate the effect of the feeding system, lairage time and temperament, on carcass and meat quality in Braford and Hereford steers.

2. Materials and methods

This study was run by the National Institute of Agricultural Research at INIA Tacuarembó Research Station, Uruguay (Latitude South 32° 02' 12.4"; Longitude West 57° 09' 15.2") over a period of 7 months (December 2006 to June 2007).

2.1. Animals and diets

Sixty Hereford and Braford steers 2.5 years old were assigned to two different feeding strategies with finishing purposes, according to live weight (LW) and breed: D1) rangeland plus corn grain (1 % LW, Hereford n=15, Braford n=15) and D2) high quality pasture composed mainly by lotus (*Lotus corniculatus*) and a small proportion of white clover (*Trifolium repens*) (Hereford n=15, Braford n=15). The area for each finishing strategy was divided in two plots by electric fencing and animals alternated plots every 14 days. The system was planned in order to avoid over-grazing. Pasture height, availability, and quality were registered for each plot every grazing period (pre and post grazing). The same data was collected for the pasture remaining in each plot. Pasture results are not presented in this paper.

Chemical composition of feeds offered is reported in Table 1.

Table 1. Chemical composition of feeds offered.

	OMD (%)	CP (%)	ADF (%)	NDF (%)
Improved pasture	60.00	21.98	36.42	54.85
Native pasture	34.39	9.22	46.29	80.25
Corn grain	81.25	8.93	13.90	22.65

OMD: organic matter digestibility, CP: crude protein, ADF: acid detergent fibre, NDF: neutral detergent fibre.

2.2. Field determinations

Productivity measurements

Animals were weighed early in the morning without previous fasting every 14 days. For D1 amounts of corn grain were provided once a day early in the morning (6 AM) and were adjusted at this time according to LW. Animals from both diets had *ad libitum* access to water.

Temperament

Temperament was individually assessed each 14 days by both non-restrained and restrained tests, based on existing or adapted methodologies proposed by different authors (Burrow et al., 1988; Fordyce et al., 1982; Grandin and Deesing, 1998; Hearnshaw et al., 1979; Hemsworth et al., 1989; Le Neindre et al., 1995; Sato, 1981). The tests used were 1) *Crush score* (CS): which is the behaviour of each animal when put into a crush using a 1 (calm) to 5 (combative) scale, 2) *Flight-time* (FT): is the amount of time (in seconds) it takes an animal to cover a fixed distance (5 meters) after it leaves the restraining device and 3) *Exit speed* (ES): is the speed for leaving the squeeze chute and was ranked as 1 = walked, 2 = trotted, and 3 if the animal ran out of the chute. A multicriterial average temperament index (Average Tindex) was built with FT, CS and ES.

2.3. Transport and slaughter plant

Animals were slaughtered in the same day in a commercial abattoir licensed for exporting meat. Each slaughter group was composed of 50% of animals from D1 and 50% from D2. The slaughter point was fixed at 470 Kg average LW within each diet. Both slaughter groups were transported for 4 hours in a commercial truck with two compartments, allowing 420 Kg/m² (1-1.2 m²/head) according to protocols from the abattoir (based on international recommendations) and remained in lairage pens for 3 and 15 hours respectively. Steers from different diets (and different slaughter groups) were not mixed either in the truck or in the abattoir.

2.4 Slaughter and sampling procedures

2.4.1. Carcass traits

Carcasses were graded using the Uruguayan Grading System (INAC, 1997) based on conformation and fatness scores. Carcass conformation was based on a visual assessment of muscle mass development, with lower numbers indicating better conformation (1= good muscle development and 6= poor muscle development). The conformation score system for Uruguay is : **I(1), N(2), A(3), C(4), U(5), R(6)**. Fat finishing was based on the amount and distribution of subcutaneous fat, using a five grade scale, where lower numbers indicate lack of fat cover and higher numbers, excessive covering. The scores used in Uruguay are: **0, 1, 2, 3, 4**.

Hot carcass weight (HCW) was registered. Carcass pH and temperature were measured at 1, 3, 6, 12 and 24 hours *post mortem* (pm) at the *Longissimus dorsi* (LD) muscle between 12-13th rib, using a thermometer (Barnant 115) with type E thermocouple and pHmeter (Orion 210A) with gel device. Subcutaneous fat thickness (SFT) and instrumental fat colour were recorded at 48 hours pm, the last based on L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) colour space, using a colorimeter (Minolta C10) with an 8 mm diameter measurement area. At 36 hours pm carcasses were ribbed between 10-11th rib, obtaining primal cuts. The fabrication process was carried out according to a European commercial standard (United Kingdom). From the pistola cut, 7 boneless cuts were obtained (Striploin, Tenderloin, Rump, Topside, Silverside, Knuckle, Tail of Rump), and the weight of trimmings and bones was recorded separately. Retail cuts were weighed and retail yields were calculated. Muscle, fat and bone percentages were calculated from the pistola cut using the UK commercial standard (cuts with 5% of fat cover).

2.4.2. Meat quality

Two steaks per animal were vacuum packaged individually and transported to INIA Tacuarembó Meat Laboratory. One (1 cm thickness) was immediately frozen at -20°C and used for lipid content determination. The second (2.54 cm thickness) was aged for 7 days at 2-4 °C. Meat colour and toughness were measured after the aging period.

Instrumental colour

Meat bags were opened and the exudation on the steak surface was removed with a paper towel. Muscle colour was measured on the LD after seven days of aging at the L*, a* and b* colour space, using a Minolta C10 colorimeter with an 8 mm diameter measurement area, after one hour of blooming. Values were registered from three different locations on the upper side of the steaks in order to obtain a representative average value of meat colour.

Marbling and lipid content

Subjective marbling was determined after 1 hour of blooming in the first LD steak, by the USDA quality grade system (USDA, 1997). Total lipid content was measured by solvent extraction, based on Folch et al. (1957).

Shear force

The second LD steak was placed inside a polyethylene bag and cooked in a water bath until an internal temperature of 70°C was achieved, using a Barnant 115 thermometer with type E thermocouple. Six cores, 1.27 cm diameter, were removed from each steak parallel to the muscle fiber orientation. Shear force measurement (WBSF) was obtained for each core using Warner Bratzler (Model D 2000) and an average value was calculated for each steak.

Cooking loss

Cooking loss was determined as the percentage difference between the raw steak (pre-cooked weight) and its weight after cooking.

2.5. Statistical analysis

Exploratory analyses were performed with the Statgraphics and SAS programmes.

Productive variables. A general linear model (PROC GLM; SAS, 2007) was used to evaluate the effect of diet, lairage time, breed and temperament on ADG. All interactions were considered and when they were not significant they were removed from the model.

Temperament. A multicriterial temperament index (Average Tindex) was built with FT, CS and ES, using the Analytic Hierarchy Process (AHP) (Saaty, 1980). A higher Tindex implied a calmer animal.

Carcass traits and meat quality. A general linear mixed model was used to study the effect of diet and breed on carcass conformation and degree of finishing (PROC GLIMMIX; SAS, 2007). The interaction between diet and breed was considered and as it was not significant, it was removed from the model.

A general linear model (PROC GLM; SAS, 2007) was used to evaluate the effect of diet, lairage time, breed and temperament on carcass and meat quality traits. Considering differences in initial and final LW between diets, both variables were included in the model as covariates. All interactions were considered and when they were not significant they were removed from the model. A regression analysis was performed to study the effect of different variables and factors on meat tenderness (PROC REG; SAS, 2007). In order to study the direct relationship between tenderness and temperament, shear force values were adjusted by all factors and variables that could have an effect on it (pH, slaughter group, breed, initial

and final live weight). Residuals from this regression were plotted with temperament and a regression analysis was performed with these two variables. Several correlation analyses were performed between productive, physiologic and temperament-related data. Means were compared by the LSMEANS procedure (SAS, 2007).

3. Results and discussion

3.1. Productivity and temperament

ADG did not differ between finishing strategies (0.63 ± 0.02 in D1 and 0.64 ± 0.02 in D2) but due to differences in initial LW, animals from D1 showed lower final LW than those from D2 (436.03 ± 45.43 and 468.46 ± 34.76 Kg, respectively). In spite of these differences and considering the experiment's main objectives, all animals were slaughtered on the same day. Pasture consumption was not restricted in any diet, crude protein contents were above critical values (6 %) and energy restrictions in D1 were released with the energetic supplementation, allowing animals to achieve the targeted daily gains.

Average Tindex did not differ between finishing strategies but breed had a significant effect on it ($p<0.05$). Braford animals were more excitable, showing a lower Average Tindex than Hereford steers (62.10 ± 4.10 in Hereford and 50.90 ± 4.00 in Braford). Genetic differences in tameness are well known (Manteca and de la Torre, 1996) and many authors have reported that without exception, *Bos indicus* (Burrow and Corbert, 2000; Fordyce et al., 1988) and *Bos indicus* cross breeds (Voisinet et al., 1997 a) were more excitable than *Bos taurus* cattle. Calmer animals had higher ADG within both breeds ($p<0.05$) and these results are also consistent with Voisinet et al. (1997 a), who reported higher ADG in calmer *Bos indicus*-cross and *Bos taurus*. A depression of growth is the consequence of a series of acute or chronic responses due to human presence (Barnett et al., 1983; Hemsworth et al., 1996) being more relevant with temperamental animals. In spite of being more excitable, Braford steers had higher ADG than Hereford ones (0.73 ± 0.05 and 0.53 ± 0.05 , respectively; $p<0.05$). When feed is not restricted, Braford animals usually have better ADG because they have a higher potential for muscle accumulation and their consumption is higher per unit weight. The use of synthetic breeds like Braford presents the heterosis advantage (hybrid vigor) associated with productive traits (Dickerson, 1969; López, 2000).

3.2. Carcass traits

Animals from D1 had higher HCW and PCW than D2 (Table 2). This information is consistent to Vaz Martins et al. (2003) results who compared three feeding systems, pasture-fed animals and pasture plus grain (offered at 0.7 and 1.5 % LW), reporting that animals with grain supplementation at 1.5 % of LW showed the highest HCW and PC weight. Other

authors obtained similar results (higher HCW and PC in supplemented animals) comparing steers from pastures versus pastures plus grain offered at 1.2 % (del Campo et al., 2008) and 1% of LW (Brito et al., 2008). Striploin, tenderloin and rump (R&L) represent most of the commercial value of the carcass and some markets require a certain weight for each cut. In this experiment, the heaviest carcasses also had the highest seven boneless cuts (7C) weight and R&L weight. However, pistola cut yield (PC/half carcass weight; PCY), valuable cuts yield (7C weight/PC; 7CY) and R&L yield (R&Lweight/PC, RLY) did not differ between diets ($p<0.05$).

Table 2. Effect of diet, lairage time and breed, on hot carcass weight, pistola cut weight, seven boneless cuts weight and Rump&Loin weight.
Least square means \pm Standard error.

	Diet 1	Diet 2	Long lairage 15 hours	Short lairage 3 hours	Diet effect	Lairage effect	Breed
HCW, (Kg)	227.05 \pm 1.26	214.30 \pm 1.31	218.31 \pm 1.23	223.00 \pm 1.23	<0.05	<0.05	< 0.05
PC wt, (Kg)	50.20 \pm 0.37	47.70 \pm 0.38	48.08 \pm 0.36	49.76 \pm 0.36	<0.05	<0.05	ns
7C wt, (Kg)	29.19 \pm 0.33	27.51 \pm 0.34	27.93 \pm 0.32	28.77 \pm 0.32	<0.05	Ns	<0.05
R&L wt, (Kg)	10.04 \pm 0.08	9.44 \pm 0.09	9.67 \pm 0.08	9.82 \pm 0.08	<0.05	Ns	<0.05

Diet 1: Pasture + grain, Diet 2: High quality pasture.

HCW: hot carcass weight, PC: pistola cut, 7C: seven boneless cuts, R&L: rump and loin.

Braford animals had higher HCW, 7C and R&L weight than Hereford steers (Table 3) and also had better meat yield (7CY and RLY; $p<0.05$). Similar results were found by several authors when comparing meat yield between *Bos Taurus* and *Bos Taurus*Bos Indicus* crosses (Franco et al., 2003; Joandet et al., 1989; Wheeler et al., 1997). Meat yield differences between breeds are generally due to different fat levels. However, when animals are compared at the same fat level, these differences could be explained by the muscle/bone relation (Purchas, 2000). In this experiment, Braford animals had higher muscle percentage and lower bone percentage than Hereford in the pistola cut ($p<0.05$). Animals with a high growing potential like Braford, have later maturity and consequently they have more protein than fat deposition (Di Marco, 1994, 2004; Joandet, 1990). In general, they accumulate more energy as proteins when compared to fat accumulation (Webster, 1989). This implies a heavier slaughter weight for reaching the same finishing state (Gregory, 1982).

Table 3. Hot carcass weight, boneless cuts weight and Rump&Loin weight in Braford and Hereford steers. Least square means \pm Standard error.

	Braford	Hereford	Significance
HCW, (Kg)	222.47 \pm 1.20	218.84 \pm 1.20	<0.05
7C wt, (Kg)	29.27 \pm 0.27	27.43 \pm 0.27	<0.05
R&L wt, (Kg)	10.07 \pm 0.09	9.41 \pm 0.08	<0.05

HCW: hot carcass weight, 7C: seven boneless cuts, R&L: rump and loin.

In our experiment, lairage time also had a significant effect on HCW and PC weight (Table 2) but regarding values and variability, differences were not considered relevant. Besides and according to the literature, during the initial 24 and 48 hours of fasting, the majority of weight lost in cattle originates from excretion of gastrointestinal tract contents and urine. As the duration of food and water deprivation extends beyond 48 h, tissue catabolism and dehydration increase their contribution to liveweight loss (Ferguson and Warner, 2008). Wythes and Shorthose (1984) concluded that carcass weight loss was typically not observed until after 24 hours of food and water deprivation in cattle.

Conformation did not differ either between diets or between breeds ($p<0.05$) but carcasses from D1 had higher fat grading than those from D2 according to the Uruguayan classification system (Table 4). SFT was also higher in carcass from the supplemented treatment (7.26 ± 0.45 vs 4.51 ± 0.47 in D2; $p< 0.05$). In general, higher carcass weight produces higher muscle thickness and fat deposits, meaning that carcass and all its components have greater dimensions (Sañudo, 1997).

Hereford steers had a higher degree of finishing than Braford within the supplemented treatment ($p<0.05$), with no differences between breeds in D2 (Table 4). According to Berg and Butterfield (1976) some breeds begin fat deposition earlier than others. British breeds like Hereford and Angus (early mature breeds) are smaller and begin fat deposition at a lower live weight when compared to continental breeds and others (Brito and Jiménez de Aréchaga, 2004). This condition could be partially explained by the higher level of energy in D1. The energy level of the pasture in D2 was probably not enough to bring about fat differences between breeds. However, in our experiment, Braford and Hereford steers did not differ either in subcutaneous fat thickness (SFT) or in PC fat percentage (see following discussion).

Table 4. Conformation and degree of finishing by diet and breed (number of animals and percentage).

	Degree of finishing		Conformation			C	
	1	2	N	A	C		
D1 Braford	7 27%	10 17%	8 24%	20 34%	2 67% 3 5%	13 28% 23 39%	0 4 7%
D1 Hereford	3 12%		12 36%		1 33%	10 21%	4 40%
D2 Braford	6 23%	16 27%	9 27%	13 22%	0 0	15 33% 23 39%	0 6 10%
D2 Hereford	10 38%		4 12%		0	8 17%	6 60%

D1: Pasture + Grain, D2: High quality pasture.

Muscle percentage did not show differences between diets when PC composition was evaluated. Similar results were found by del Campo et al. (2008) who compared steers finished on pastures vs. supplemented with grain at 1.2% LW. On the contrary, many studies had reported that a higher grain or concentrate level during finishing periods leads to a lower proportion of muscle and bone in the carcass, along with a higher percentage of fat (Keane et al., 1989). Although there were no differences in muscle percentage, pistola cut from D1 had higher fat percentage than pistolas from D2. Similar results were obtained by other authors, reporting that fat proportion rises with concentrate-based diets (del Campo et al., 2008; Micol, 1993). Differences in carcass composition are mainly related to differences in the energy density of the diet and, in the end, to the total energy consumed (Cerdeño et al., 2006).

3.3. Meat quality

pH and temperature

Higher SFT probably motivated the higher carcass temperature registered in D1 at 1, 3, 6, and 24 hours pm (Table 6). However, no differences in pH decline were obtained between diets ($p < 0.05$; Table 5). pH and temperature decline showed no differences between breeds ($p < 0.05$).

On the other hand, lairage time did have a significant effect on pH values. Carcasses from the short lairage group had higher pH values at 1, 3, 6, and 24 hours pm (Table 5) suggesting that animals were more stressed than those from the long lairage. Preslaughter stress is likely to reduce muscle glycogen level in vivo (Warris, 1990) because of energy expenditure due to physical exercise or psychological stress, which may in turn increase ultimate pH of muscles (Gregory and Grandin, 1998). This depletion limits the extent of muscle/meat acidification. Warris (1990) sustains that this is mainly explained by physical

exertion associated for example, with agonistic behaviour like mounting and fights, especially in the muscles of the back and legs (*M. longissimus dorsi* and *semitendinosus*) (Tarrant, 1989; Tarrant and Sherrington, 1980; Tarrant et al., 1988). Stressors appear to be additive (Bray et al., 1989) and multiple stressors in the preslaughter period will result in a greater elevation of ultimate muscle pH than a single stressor alone.

Table 5. Effect of diet, lairage time and breed on the rate of pH decline.
Least square means \pm Standard error.

	<i>Diet 1</i>	<i>Diet 2</i>	<i>Long lairage</i>	<i>Short lairage</i>	<i>Diet effect</i>	<i>Lairage effect</i>
pH1	6.71 \pm 0.07	6.67 \pm 0.07	6.56 \pm 0.06	6.82 \pm 0.06	ns	<0.05
pH3	6.50 \pm 0.07	6.48 \pm 0.07	6.24 \pm 0.06	6.74 \pm 0.06	ns	<0.05
pH6	6.11 \pm 0.05	6.21 \pm 0.05	6.02 \pm 0.05	6.30 \pm 0.05	ns	<0.05
pH24	5.75 \pm 0.03	5.75 \pm 0.03	5.67 \pm 0.03	5.83 \pm 0.03	ns	<0.05

Diet 1: Pasture + grain, Diet 2: High quality pasture.

Table 6. Effect of diet, lairage time and breed on the rate of temperature decline.
Least square means \pm Standard error.

	<i>Diet 1</i>	<i>Diet 2</i>	<i>Long lairage</i>	<i>Short lairage</i>	<i>Diet effect</i>	<i>Lairage effect</i>	<i>Diet * lairage</i>
Temp 1	33.80 \pm 0.40	32.30 \pm 0.42	29.30 \pm 0.39	36.80 \pm 0.39	<0.05	<0.05	<0.05
Temp 3	26.10 \pm 0.39	22.40 \pm 0.40	24.90 \pm 0.38	23.60 \pm 0.38	<0.05	<0.05	<0.05
Temp 6	16.70 \pm 0.29	13.80 \pm 0.30	17.70 \pm 0.28	12.80 \pm 0.28	<0.05	<0.05	<0.05
Temp 24	3.20 \pm 0.08	2.70 \pm 0.08	2.80 \pm 0.08	3.10 \pm 0.08	<0.05	<0.05	ns

Diet 1: Pasture + grain, Diet 2: High quality pasture.

In this study, overnight animals appeared to recover during waiting time and this was reflected on a better rate of pH decline and also on meat tenderness (see shear force discussion further on). Time spent in lairage probably allowed cattle to replenish muscle glycogen concentrations and to rest and recover from some of the previous stressors. According to Warriss et al. (1984) glycogen resources can be restored at lairage, and cattle can recover from physical exhaustion even if they are not fed. Similar results were found by Mounier et al. (2006) who evaluated 3 main waiting times in final pH: 1, 17, or 40 h before slaughter, reporting that pH decreased accordingly. On the other hand, several authors

indicate that the environment itself may inhibit the ability of cattle to rest or recover from the effects of feed and water restriction (Jarvis et al., 1996; Van de Water et al., 2003). Inconsistencies among authors could be explained not only by the lairage duration evaluated in each experiment but also by differences in animal experience (handling and feeding system), temperament and breed (genetic affiliation), handling procedures from farm to death, transport duration and conditions, facilities of the abattoir, weather conditions, and/or cumulative effects of the different factors. In this study, the negative effect of lairage probably occurred in the short lairage group mainly because animals did not have enough time to get used to the new environment. It is worth noting that 55% of animals from the short lairage group, showed pH values higher than 5.8.

Temperament was related neither to initial nor to final pH. Similarly, no association between temperament and the incidence of ultimate pH was observed in the studies carried out by Fordyce et al. (1988) and Petherick et al. (2002).

Fat and meat colour

Fat colour was not affected by diet, breed or lairage time. Cattle produced under extensive grass-based production systems generally have yellower carcass fat than their intensively-reared, concentrate-fed counterparts, caused by carotenoids from green forage (Dunne et al., 2009). In our experiment, the grain level used in the supplemented treatment was not enough to diminish fat yellowness ($b^*= 19.70 \pm 0.40$ and 18.90 ± 0.40 , in D1 and D2, respectively). These results are not consistent with those reported by del Campo et al. (2008) who indicated higher fat b^* values in pasture fed-animals, as compared to supplemented ones (corn grain at 0.6 and 1.2 % of LW). However, the absolute fat b^* values from the present experiment were similar to those reported by the above-mentioned authors in the pasture treatment ($b^*=18.80 \pm 0.40$).

Muscle colour measured after 7 aging days was not affected either by diet or breed (Table 7). Other researchers did not find significant effects of different forage/concentrate ratio diets during finishing on muscle colour (Brito et al., 2008; Cerdeño et al., 2006; French et al., 2001). Diet characteristics do not have capital relevance on meat colour, probably due to transformation processes that take place in the rumen (Albertí et al., 1992; Hedrick et al., 1983). In our experiment, redness and b^* values were higher in the long lairage group (Table 7). Lower pH values (long lairage group) were linked to more red (higher *a) and yellow (higher *b) meat. It has been established by several authors that muscle colour is highly correlated with muscle pH (Page et al., 2001; Wulf and Page, 2000). Page et al. (2001) reported that a^* and b^* values were more highly correlated with muscle pH ($r= -0.58$ and -0.56 , respectively) than L^* values ($r= -0.40$). In our experiment a^* and b^* values were also

more significantly ($p<0.05$) correlated to pH3 ($r= -0.33$, a^*), pH6 ($r= -0.59$ and -0.48 , a^* and b^* , respectively) and pH24 (-0.40 , b^*).

Table 7. Effect of diet and lairage time on muscle colour.

Least square means \pm Standard error.

	Diet 1	Diet 2	Long lairage	Short lairage	Diet effect	Lairage effect
L*	39.50 \pm 0.35	39.10 \pm 0.36	39.80 \pm 0.34	38.90 \pm 0.34	ns	ns
a*	9.90 \pm 0.27	9.60 \pm 0.27	10.20 \pm 0.26	9.40 \pm 0.26	ns	<0.05
b*	10.20 \pm 0.15	10.10 \pm 0.15	10.40 \pm 0.14	9.90 \pm 0.14	ns	<0.05

Diet 1: Pasture + grain, Diet 2: High quality pasture.

Marbling and lipid content

Subjective marbling and lipid content did not differ either between diets or between breeds ($p<0.05$). These results were consistent with those reported by Schindler et al. (2004) who did not find differences in marbling when grain was included in the diet of steers compared at the same finishing state. In this experiment, all carcasses were in the USDA “Slight” category. With regard to lipid content, least square means and standard errors were $2.10 \pm 0.10\%$ in D1 and $1.94 \pm 0.10\%$ in D2. Similar results were found by Brito et al. (2008) in a study that also included Braford and Hereford animals, registering 2.3 and 1.9 % of lipid content in pastures and supplemented-fed steers, respectively, with no significant differences either between diets or between breeds.

Shear force and cooking loss

Finishing strategy did not have an effect on WBSF values (Table 8). These results are consistent with those reported by Brito et al. (2008) evaluating pastures vs. supplement-fed steers (1 % LW).

Table 8. Effect of diet, lairage time and breed on meat shear force.

Least square means \pm Standard error.

	Diet 1	Diet 2	Long lairage	Short lairage	Diet effect	Lairage effect	Breed
WBSF (Kg F)	5.60 \pm 0.39	5.10 \pm 0.40	4.30 \pm 0.38	6.40 \pm 0.38	ns	<0.05	<0.05

Diet 1: Pasture + grain, Diet 2: High quality pasture.

WBSF: Warner Bratzler shear force.

Meat from animals in the long lairage group showed lower WBSF values than steers from the short one (Table 8). According to Kauffman and Marsh (1987) glycolytic rate in the first few

hours post slaughter is a major determinant of quality. The pre-rigor muscle environment is critical in determining the behaviour of myofibrillar proteins and their subsequent impact on meat quality attributes such as tenderness and colour. According to Dransfield (1994), the effects of calpains and their inhibitors immediately *post mortem*, depend on pH and temperature and have an important influence on tenderness (review: Koohmaraie, 1996). Muscle pH and temperature also interact continuously during rigor development as they impact on both physical shortening and proteolytic enzyme activity. In our experiment, differences in pH and temperature rate decline (1, 3 and 6 hours) had already probably determined differences between the two slaughter groups in meat tenderness. Correlations between WBSF and pH values partially confirm this ($\text{pH}1=0.56$, $\text{pH}3=0.55$, $\text{pH}6=0.47$; $p<0.05$).

We analysed the short lairage group in depth in order to determine the specific causes of the high shear force values registered in this group. As mentioned, depletion of muscular glycogen reserves because of greater pre slaughter stress, without the opportunity of resting and restoring energy reserves, probably had a considerable influence on pH values in carcasses from these animals. Although fat thickness reduced temperature decline and increased pH decline rate in D1, it had no consistent effects on shear force values which were not significantly different (6.10 ± 0.62 and 6.70 ± 0.65 , in D1 and D2, respectively). However, it is worth noting that D2 carcasses from the short lairage group, presented conditions on the borderline of cold shortening (10.1°C and pH 6.45 at 6 hours). Very similar results were found by Koohmaraie et al. (1988) studying the role of subcutaneous fat as an insulator of beef carcasses. They reported that fat thickness reduced temperature and increased pH decrease, with no consistent effects on shear force values. However, when the fat cover was removed from sides, they presented conditions near cold shortening (10.8°C and pH 6 at 6 hours *pm*). In this study, there were no significant differences between diets in shear force in the 4 hour-group but WBSF values around 10 and 11 in both breeds after 7 aging days suggested that cold shortening had probably occurred in some carcasses. Shear force correlation coefficients with temperature at 6 hours *pm* partially confirms our theory ($r=-0.4$, $p<0.05$).

In this experiment, Braford steers had higher WBSF values than Hereford (5.93 ± 0.32 vs. 4.78 ± 0.32 respectively). Meat from *Bos indicus* breeds of cattle is often less tender than meat from *Bos taurus* (Crouse et al., 1987, 1989; Koch et al., 1982; McKeith et al., 1985). Genetic differences are partly due to a reduced post-mortem proteolysis of myofibrillar proteins in *Bos indicus* associated with a higher activity of calcium dependent protease inhibitor (Whipple et al., 1990). Considering that breed did not have a significant effect on pH decline and final pH values, breed differences in tenderness in our study were not

attributable to stress derived from the experimental conditions, but were probably mainly explained by the known genetic factors, in other words, differences in calpastatin activity in the meat (Kooohmaraie, 1995). It is worth noting that shear force values for Braford animals in this study were higher than those reported by Brito and Pittaluga (2002) after 7 aging days (WBSF: 4.52 ± 1.09) and by O'Connor et al (1997), who reported values of 3.18 after the same aging period.

Shear force was significantly related to temperament ($p<0.05$), implying that calmer animals (higher Average Tindex) had lower shear force values with 7 aging days. It is well known that stress impairs the meat aging process, generally leading to tougher meat (Ouali et al., 2006). Considering there was no association between temperament and pH as previously mentioned, our results suggested that temperament did have an effect on tenderness, independently of its effect through the rate of pH decline. One possible explanation could be through the anti-apoptotic activity of heat shock proteins (HSPs). When animals are under intense stress, the cells receive the apoptosis-inducing signals via the receptors of cellular death. If the stress is not as severe, cells prepare their defense by synthesis of HSPs for helping in the protection of intracellular components and structures against hazards associated with loss of their biological functions (Beere, 2004). This condition would be accentuated in more temperamental animals. Consequently, the process of cellular death will be slowed down and this will constitute an impediment to good meat ageing (Ouali et al., 2006). In addition, the altered metabolism associated with greater stress responsiveness in the more stressed and excitable cattle may have created conditions that were less favorable to calpain-mediated proteolysis (King et al., 2006).

Diet, lairage time and breed did not have an effect on cooking loss percentages at 7 days post-slaughter ($p<0.05$). Similar results were found by other authors when comparing carcasses from diets with different forage and grain-concentrate rates (Cerdeño et al., 2006; Kerth et al., 2007).

4. Conclusions

Finishing diets did not determine differences in ADG, but calmer animals had higher ADG and lower shear force values within both breeds. Carcass, pistola cut, 7 boneless cuts and R&L weights were higher in D1, but meat yield did not differ between diets. Fat percentage in the pistola cut and fat thickness were higher in D1 with no deleterious effects on retail cuts and valuable boneless cuts. Meat quality did not differ between diets, but short time in lairage had a significant negative effect on pH values and meat tenderness. The first few hours post-slaughter are critical in determining glycolytic rate and the behavior of myofibrillar proteins,

with the subsequent impact on meat quality attributes such as tenderness. According to this experiment, more than 3 hours in lairage pens are necessary to allow animals to habituate to the new environment and to rest, and probably to allow muscle glycogen repletion in cattle. Braford steers had higher carcass weight, valuable cuts and lean yield than Hereford, but the animals were more excitable and showed higher shear force meat values.

5. Implications

The addition of low level of grain in a pasture finishing diet appears to improve carcass weight with no deleterious effect on fat and meat quality. Special consideration should be paid to lairage time with regard to pH and temperature decline, and consequently shear force values, especially under stressful pre-slaughter conditions. Temperament appears to be an important tool regarding its effect on productivity and also on meat quality.

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CAPÍTULO VII - ARTÍCULO 4

CAPÍTULO VII - Animal welfare related to temperament and different pre slaughter procedures in Uruguay⁶

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Abstract

Sixty Hereford (H) and Braford (B) steers were assigned to two diets: D1) native pasture plus corn grain (1 % of live weight) (H n=15, B n=15); and D2) high quality pasture (H n=15, B n=15) for finishing purposes. Temperament was individually assessed and monitored during the experiment. All animals were slaughtered the same day in two groups (50 % of animals from D1 and 50 % from D2 in each group) after staying 15 and 3 hours in lairage pens, respectively. Different physiological indicators were used to assess stress after transport, lairage and immediately preslaughter. Carcass quality was determined through the incidence of bruising and final pH. Calmer animals had higher average daily gains (ADG) with no differences between diets. Transport was not a psychological stressful stage but animals were physically affected. The group that remained 3 hours in lairage pens showed a higher frequency of negative behaviour. These stressed animals did not have enough time to cope with the environment, with the consequent deleterious effects on final pH. The long lairage group had a higher metabolic response but these animals could rest and recover, and reached adequate final pH values. Braford steers were more excitable during the finishing period and also during lairage. Regardless of breed, temperament appears to be a valid tool for increasing productivity and decreasing the physiological stress response during all preslaughter stages. Further research should be carried out to establish the proper intermediate lairage duration according to animal welfare, and carcass and meat quality criteria.

Keywords: lairage time, stress response, temperament, transport in cattle

1. Introduction

Transport and handling of slaughter animals are associated with a series of events which cause stressful and unfavourable conditions, compromise animal welfare, increase the

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chance of spreading disease (Gebresenbet and Eriksson, 1998; Gross and Siegel, 1993) and reduce meat quality (Honkavaara and Kortesniemi, 1994; Warriss et al., 1995).

Strict regulations and directives have been issued to promote animal welfare during transit and the significant relationship between pre-slaughter stress and meat quality has been widely documented (Ferguson et al., 2001). However, adequate scientific data are lacking on stress-inducing factors in cattle transport and lairage. In many European countries and North America it is common to slaughter animals on the day of arrival, whereas in Australia, New Zealand and Uruguay, animals are more typically slaughtered the day after arrival. Several authors sustain that time lairage potentially allows cattle to replenish muscle glycogen concentrations, reduce dehydration of body tissues and carcass weight loss and to rest and recover from the effects of transport. Other authors think that the lairage environment itself may inhibit the ability of cattle to rest or recover from the effects of feed and water restriction (Jarvis et al., 1996).

The magnitude and quality of the stress response will be greatly affected by individual differences (Moberg, 1985). Animals with the most excitable temperaments may be most susceptible to stress generated by routine handling practices, such as loading and unloading, transport or the new environment in the abattoir (Lensink et al., 2000) and these differences will be reflected in physiological indicators. Stress may activate the pituitary-adrenocortical system (Rijnberk and Mol, 1989) and these hormonal changes may affect cellular metabolic processes (Terlouw et al., 2005). Previous research has indicated that cattle with more excitable temperaments had more extensive responses to a simulated stress challenge and higher basal concentrations of glucocorticoids (Curley, 2004). This suggests that stress response mechanisms are much more active in excitable animals than in their calmer counterparts. By choosing appropriate physiological measurements of psychological and physical stress in conjunction with behavioural observations of animals in slaughterhouses, it is possible to make an initial assessment of the effect of being restrained, the new environment, feed and water restrictions, as well as other kinds of stress associated with transport and pre-slaughter management, on the welfare of cattle (Moss, 1984).

The objective of this experiment was to evaluate the effect of animal temperament and different preslaughter procedures, on animal welfare in Braford and Hereford steers.

2. Materials and methods

2.1 Animals and diets

This study was run by the National Institute of Agricultural Research at INIA Tacuarembó Research Station, Uruguay (Latitude South 32° 02' 12.4"; Longitude West 57° 09' 15.2") over

a period of 7 months (December 2006 to June 2007). Sixty Hereford and Braford steers 2.5 years old were assigned to the following two feeding strategies with finishing purposes according to live weight, horn presence and breed: D1) rangeland plus corn grain with the grain supplied at 1 % of live weight (LW) (Hereford n=15, Braford n=15); and D2) high quality pasture composed mainly of lotus (*Lotus corniculatus*) with a small proportion of white clover (*Trifolium repens*) (Hereford n=15, Braford n=15). The area for each finishing strategy was divided into two plots by electric fencing and animals alternated plots every 14 days. The system was planned in order to avoid over-grazing.

2.2. Field determinations

Productivity measurements

Animals were weighed early in the morning without previous fasting, every 14 days. For D1, amounts of corn grain were adjusted at this time according to LW. The supplement was provided once a day early in the morning (6 AM). Animals from both finishing strategies had *ad libitum* access to water.

Tameness and temperament

Tameness and temperament were evaluated by non-restrained and restrained tests based on existing or adapted methodologies (Burrow, 1988; Fordyce et al., 1982; Grandin and Deesing, 1998; Hemsworth et al., 1989; Le Neindre et al., 1995). The Flight zone (FZ) is the animal's personal space and its size depends on the tameness of livestock. It was registered in each finishing strategy 2 days before slaughter. The test was always performed by the same person, who slowly walked (2 steps/second) toward the group of animals, registering the distance (in meters) when half of them turned away. Hair whorl position (HWP) was recorded on the first day of the experiment, looking for a correlation with temperament. If the center of the hair whorl was above the top of the eyes, the animal was categorized as "excitable"; "medium" if the center was located at eye level and "calm" if the center was located below the bottom of the eyes (Grandin et al., 1996). Temperament ratings were assessed individually every 14 days by the following 3 tests: 1) *Crush score* (CS): is the behaviour of each animal when put into a crush using a 1 (calm) to 5 (combative) scale, 2) *Flight-time* (FT): the amount of time (in seconds) it takes an animal to cover a fixed distance (5 meters) after it leaves the restraining device and 3) *Exit speed* (ES): the speed on leaving the squeeze chute and was ranked as: 1 = walked, 2 = trotted, and 3, if the animal ran away from the chute. A multicriterial temperament index (Tindex) was built from FT, CS and ES.

Health status

Pathological event or trauma and the corresponding medical treatments were daily observed and registered throughout the entire experimental period.

2.3. Transport and slaughter plant

All animals were slaughtered on the same day in a commercial abattoir licensed to export meat, following standard animal welfare procedures. Each slaughter group was composed of 50 % of animals from D1 and 50 % from D2, remaining in pens for 3 and 15 hours pre-slaughter, respectively. Animals were transported for 4 hours in a commercial truck with two compartments, allowing 420 Kg/m^2 ($1-1.2 \text{ m}^2/\text{head}$) according to the abattoir protocol (based on international recommendations). Steers from different diets within each slaughter group were not mixed either in the truck or in the abattoir. The same truck and driver were used for both journeys. Distance from the farm to the slaughter house was 140 km. Average driving time was 3 hours and a half, with 3 stops of 3-4 minutes for animal monitoring. No problems were registered during loading and unloading, this being fluid in both groups. After arriving at the abattoir, animals from each diet ($n=15$) within each slaughter group were taken to a 37.5 m^2 pen with 2 divisions (8 and 7 animals per division). The space allowance in lairage pens was $420 \text{ Kg}/2.5 \text{ m}^2$, according to the protocol mentioned above.

Physiological indicators

Three blood samples were taken from all animals looking for basal values in welfare indicators and their respective changes, according to the following periods:

- before leaving the farm (basal values), *Time A*
- immediately after arriving at the slaughter house (transport effect), *Time B*
- after lairage (lairage effect), *Time C*
- during bleeding (effect of the last handling procedures), *Time D*

One of these samples was collected into anticoagulant, cooled and immediately sent for hematocrit determination. The other 2 samples were kept cool until they arrived at the laboratory. Serum was extracted following centrifugation at 3000 rpm for 15 minutes. The serum fractions were frozen and immediately sent for analysis:

Sample 1 *Hematocrit* was determined by the micro hematocrit technique in the Veterinary Faculty, Uruguay. Results are expressed in percentages.

Sample 2. *Cortisol and Creatine kinase (CPK)*. Serum samples were assayed in the Nuclear Techniques Laboratory, Veterinary Faculty, Uruguay.

- Cortisol was determined by a direct solid-phase radioimmunoassay (RIA) using DPC kits (Diagnostic Product Co., Los Angeles, CA, USA). All samples were determined in the same assay. The RIA had a sensitivity of 8.2 nmol l^{-1} . The intra-assay coefficients

of variation for low (36 nmol l^{-1}), medium (224 nmol l^{-1}) and high (427 nmol l^{-1}) controls were 10%, 6.8% and 4.6%, respectively. Results are expressed in nmol/L.

- CPK. Method: CK NAC liqui UV. Liquid test for creatine kinase determination (EC 2.7.3.2.) activated by NAC and measured by spectrophotometry at 340 nm. Results are expressed in U/L.

Sample 3. *Non esterified fatty acids (NEFA) and β -hidroxibutirrate (β HB).* Serum samples were assayed in the Rubino Laboratory, Uruguay.

- NEFA. Method: ACS-ACOD (acil-CoA sintetasa-acil-CoA oxidasa). WAKO laboratory kits were used (refs. 999-34691, 995-34791, 991-34891 y 993-35191) - lots TK 365, TK 366, TK 367 y TK 368. This method was adapted for use in a VITALAB Selectra 2 Autoanalyzer. Results are expressed in nmol/L.
- β HB. Method: D-3-hidroxybutyrate oxidation into acetoacetate through the 3-hidroxibutirrate dehydrogenase enzyme. As a consequence, NAD⁺ from the reactive is reduced to NADH and absorbance change to 340 nm. RANDOX laboratory kits were used (ref. RB 1008) - 094293 in a VITALAB Selectra 2 autoanalyzer. Results are expressed in nmol/L.

Behaviour

Cattle behaviour was evaluated by 8 trained observers working in pairs, who rotated between divisions each hour. Direct observation was performed within each division (experimental unit) using a scan sampling technique (Martin and Bateson, 1993). Due to operative restrictions, animals were observed for 1.5 hours in the short lairage and 7 hours in the long lairage group. At each scan, the following states/events were recorded: walking, ruminating, lying, standing (without rumination), drinking, negative behaviour, social behaviour, and self grooming. Results are shown as the percentage of time spent performing the behaviour (related to the observation period). Fighting and mounting are considered significant behaviours, for which it is important to record each occurrence and this type of behaviour would tend to be missed by scan sampling (Martin and Bateson, 1993). In this experiment, conflicts (fighting and mounting) were also registered with the behaviour sampling technique at each pen division, between 2 scan periods. Each consecutive sample interval took 7.5 minutes. Animals were individually identified with a number painted on both sides of the body.

2.4. Carcass traits

Before carcasses were dressed they were visually inspected, recording the number and severity of bruises at the individual level. Severity was scored as major or minor, depending

on whether or not they involved tissue remotion (minor: subcutaneous or no tissue remotion; major: affecting muscle). Carcass pH was measured at 24 hours *post mortem* (pm) at the *Longissimus dorsi* (LD) between 12-13th ribs, using a pHmeter (Orion 210A) with a gel device.

2.5. Statistical analysis

Exploratory analyses were performed for all variables using Statgraphics and SAS packages.

Productivity. A general linear model (PROC GLM; SAS, 2007) was used to determine the effects of diet, horns presence, hair whorl position, breed and temperament on ADG. Initial and final liveweight were included in the model as covariates. Interactions were considered and if not significant, were removed from the model.

Tameness and temperament. A multicriterial temperament index (Tindex) was built with FT, CS and ES. A standardised ranking was created for each variable in a 1-100 scale. Tindex was created by: $Tindex = \sum W.d$, where "W" is the weight assigned to each variable estimated by the Analytic Hierarchy Process-AHP (Saaty, 1980); and "d" is each individual record, standardised (within each variable). Considering that FT is an objective test, it was assigned a relatively higher ranking in the index. The higher Tindex, the calmer the animal. For several analysis an Average Tindex was constructed, including variations in temperament during the whole period. A general linear model (PROC GLM; SAS, 2007) was used to evaluate the effect of diet, breed, horn presence and hair whorl position on Average Tindex. In order to study the effect of the diet and breed on Tindex through time (5 consecutive dates), an analysis of variance was carried out using a mixed model with repeated measures considering the animal as a random effect inside each diet. Initial and final liveweight were included in the model as covariates (PROC MIXED; SAS, 2007). When interactions between independent variables were not significant they were removed from the model.

Physiological data. Due to absence of normality, cortisol and CPK values were normalized by taking a natural logarithm. The effects of diet, breed and temperament on physiological indicators through time (4 consecutive occasions) were evaluated through analysis of variance using a mixed model with repeated measures considering the animal as a random effect inside each diet (PROC MIXED; SAS, 2007). Initial and final liveweight were included in the model as covariates. Each animal was bled on four consecutive occasions. To model the correlation between repeated measures for each animal, a general linear mixed model was used (PROC MIXED, SAS 2007). For each dependent variable analysed, several covariance structures were tested (variance components [VC], first-order autoregressive structure [AR (1)] and compound symmetry [CS]), in order to fit the best model. Goodness of fit was defined by the lower Akaike's Information Criteria (AIC) value (Akaike, 1974). After

each model had been adjusted, robustness was tested excluding from data standardised, residual values higher than 2 and lower than -2. The model was considered robust when explanatory variables stayed in the model after data filtering and model rerunning. A regression analyses was performed to evaluate Average Tindex, lairage duration and final liveweight effects, on cortisol concentration during slaughter.

Behavioural data: Binomial data from each activity was modeled assuming a binary distribution and a logit link function, using the pen division as the subject/experimental unit. A General linear mixed model was used to study the effect of lairage time, diet, breed and temperament, on the frequency of each behaviour (PROC GLIMMIX; SAS, 2007). Mounting&fighting data from the *Behaviour technique* was modeled and a log link function was set, assuming a Gamma distribution. A General linear mixed model was used to study the effect of diet, breed and temperament on fighting in the first hour in lairage (PROC GLIMMIX; SAS, 2007). Hypothesis tests (Binomial proportion) were performed to analyze differences in fighting frequency (number of events per hour) between consecutive and non consecutive hours in lairage.

Carcass quality. Bruising frequency was compared by the χ^2 test (PROC FREQ; SAS, 2007) and regression analysis were performed to study the effect of independent variables on bruising frequency (PROC LOGISTIC; SAS, 2007) and pH values (PROC REG; SAS, 2007).

3. Results and discussion

3.1. Field determinations

Productivity

ADG did not differ between diets (0.63 ± 0.02 in D1, 0.64 ± 0.02 in D2; $p<0.05$). The crude protein (CP) content of Uruguayan rangeland pastures seems not to be restrictive for animal production (Rovira, 1996) covering cattle maintenance requirements (Carámbula, 1996) but low ADG especially in autumn, are usually due to the unbalanced chemical composition of native pasture, with low energy availability for the digestive process (Andreó et al., 2001). In our experiment, grazing was not restricted in any diet, CP contents were above critical values (9.22% and 22% in D1 and D2, respectively) and energy restrictions in D1 were compensated by the energetic supplementation, providing the animals in this diet with adequate daily gains. Horn presence and HWP were not related to daily gains ($p<0.05$).

Tameness and temperament

The D1 Flight zone was lower at the end of the experimental period (0 vs 8 meters in D2). In more intensive systems the Flight Zone is necessarily reduced as compared with an open

range system (Phillips, 1993; Uetakee al., 2002). However, the D2 Flight Zone (8 meters) also implied that all the animals got used to the presence of humans. Average Tindex was not affected by diet, but breed did have a significant effect on it ($p<0.05$). Braford animals were more excitable showing a lower Average Tindex than Hereford steers (62.10 ± 4.10 in Hereford and 50.90 ± 4.00 in Braford). Based on a review of temperament of beef cattle, Burrow (1997) reported that without exception, *Bos indicus* were more excitable, temperamental and difficult to handle under extensive management conditions than *Bos Taurus* breeds (Burrow and Corbet, 2000; Elder et al., 1980; Fordyce et al., 1988; Hearnshaw and Morris, 1984; Powell and Reid, 1982). Regarding *B. indicus*-cross cattle, Voisinet et al (1997) reported that they also had higher mean temperament scores than *B. taurus*. Although these differences could be partly due to environmental factors, genetic differences in tameness cannot be ruled out (Manteca and de la Torre, 1996).

Braford steers had higher ADG than Hereford (0.73 ± 0.05 and 0.53 ± 0.05 , respectively; $p<0.05$) and calmer animals had higher ADG within both breeds ($p<0.05$). These results are consistent with those of Voisinet et al. (1997), who reported that calmer *Bos indicus*-cross and *Bos Taurus* cattle had higher ADG than steers with excitable temperaments. Barnett, et al. (1983) and Hemsworth et al. (1996) sustained that a fall in the rate of growth is the consequence of a series of acute or chronic responses to human presence, and is probably more accentuated in temperamental animals. Regardless of temperament, gentler animals are known to be less susceptible to stress generated by management practices in which human presence is involved (Boivin et al., 1994) and their productivity is therefore less affected. In this study, all animals had been subjected to good animal husbandry practices prior to and during the test, which probably contributed to the satisfactory ADG levels obtained.

Average Tindex was not related to horn presence ($p<0.05$). These results are not consistent with those of Kilgour and Scott (1959) who reported that dairy cows that had not been dehorned created more trouble while being milked. These cows may have been dominant by virtue of their horns. According to Grandin et al. (1996) animals from *B. Taurus* and *B. indicus* * *B. taurus* breeds could be classified as temperamental, medium or calm according to the HWP. This relation may be explained by the fact that hair patterns in the foetus form at the same time as the brain (Smith and Gong, 1974). However, in our experiment, temperament was not related to HWP. Similar results were found by Randle (1998) who reported that cattle response to humans in general was not significantly associated with HWP.

When comparing initial and final Tindex within each diet, it was observed that animals from D2 were more excitable at the end of the experiment (Table 1). Even thought a strict protocol

of good management practices was followed during the experiment, the degree of human contact was lower in D2 because these animals were not supplemented. Habituation to humans is considered to reduce the magnitude of the stress response arising from restraint and handling (Grandin, 1997). In D1 animals did not become calmer, but their reaction to handling remained stable (Table 1).

Table 1. Initial and Final Tindex by diet (and by breed within each diet). Least square means \pm Standard error.

	D1		D2	
	Pasture + Corn Grain	High Quality Pasture	Braford	Hereford
Initial per breed	52.04 ^{b,c} \pm 7.6	65.4 ^{abc} \pm 7.7	68.5 ^{ab} \pm 7.7	76.6 ^a \pm 7.8
INITIAL	58.7 ^B \pm 5.4		72.56 ^A \pm 5.6	
Final per breed	62.01 ^{abc} \pm 7.7	60.5 ^{abc} \pm 7.9	44.2 ^c \pm 7.7	63.9 ^{abc} \pm 8.0
FINAL	61.2 ^B \pm 5.6		54.1 ^B \pm 5.6	

^{a,b,c} Values in lines and columns with different letters are different p<0.05

^{A,B} Values in lines and columns with different letters are different p<0.05

The increased excitability registered in D2 was mainly due to Braford steers (Table 1). Their reaction to handling increased with time in this diet, while Hereford reaction was stable throughout the experiment. Within the supplemented strategy, temperament evolution did not differ between Hereford and Braford steers (Table 1). As previously mentioned, there are several reports of breed differences in temperament, especially between the two cattle subspecies *Bos indicus* and *Bos Taurus*. Furthermore, within the British breeds, some authors reported that Herefords were the most docile (Stricklin et al., 1980; Tulloh, 1961). Frequent and proper handling therefore, appears to be even more important when working with more excitable animals like *Bos indicus* or its crosses. There was no difference in Final Tindex between finishing strategies (Table 1).

Health status

Health was compromised in 2 animals from D2 in the course of the experiment and no event was related to feed problems. In both cases, immediate and effective control measures were applied, with no incidence on ADG in the animals involved. Good physical health is undoubtedly a necessary condition for the well-being of animals. However, health is more than the absence of disease, and understanding the relationship between health and welfare depends on drawing inferences about subjective feelings such as pain, discomfort and distress (Appleby and Hughes, 2005). Based on productive and behavioural observations, it

was considered that these events did not have a strong negative impact on welfare and both animals remained in the experiment. No deaths were registered during the experimental period.

3.2 Transport and slaughter plant

Physiology

Cortisol, CPK and NEFA

Finishing strategy did not have an effect on physiological indicators (except β Hb). According to the genetic affiliation of steers, differences were found only for hematocrit, but all values were considered normal according to the literature. The physiological responses to water deprivation for periods of up to 48 hours in cattle and sheep generally indicate that ruminants can cope with this challenge (Ferguson and Warner, 2008). The results of the present experiment showed that differences in the diverse physiological indicators were mainly due to lairage duration and animal temperament.

Transport effect on cortisol

Figure 1 shows that each step (except transportation, Time B) involved higher stress in both slaughter groups. According to several authors, the major factors determining the well-being of cattle during road transport are: vehicle design, stocking density, ventilation, the standard of driving and the quality of the road (Broom, 2003; Hartung, 2003; Tarrant and Grandin, 1993). In our experiment, all these factors were standardised and optimised and, added to the calming role of animals herd-mates during transport, they could have been effective and could have contributed to our results. Similar results were reported by Ishiwata et al. (2008) who did not find differences in plasma cortisol concentration before and after travelling, suggesting that transport had no severe effects on cattle. Fazio et al. (2005) suggested that the effects of short-distance road transport on the increase in cortisol levels in cattle, probably depend on preliminary contact with staff during handling. Trunkfield and Broom (1990) reported a sharp response in cortisol levels in calves during the first 2 hours of transport, mainly due to the loading procedure. These authors suggested that cattle are stressed during the initial period of transportation (on short journeys), and that the degree of stress is greater after long-distance road transport. Villaroel et al (2003) also reported that cortisol was higher after 1-2 hours of transportation compared to journeys that were less than 1 hour or more than 2 hours long. After this initial period on short journeys (less than 4 hours), animals are thought to become accustomed to the new situation. In our experiment, animals from both slaughter groups showed a good habituation to transport (Figure 1, Times A and B). On the basis of the comparative response of circulating levels of cortisol before and after transportation, our data do not agree with results that consider transport to be one

of the most potent stressors for cattle (Marahrens et al., 2003).

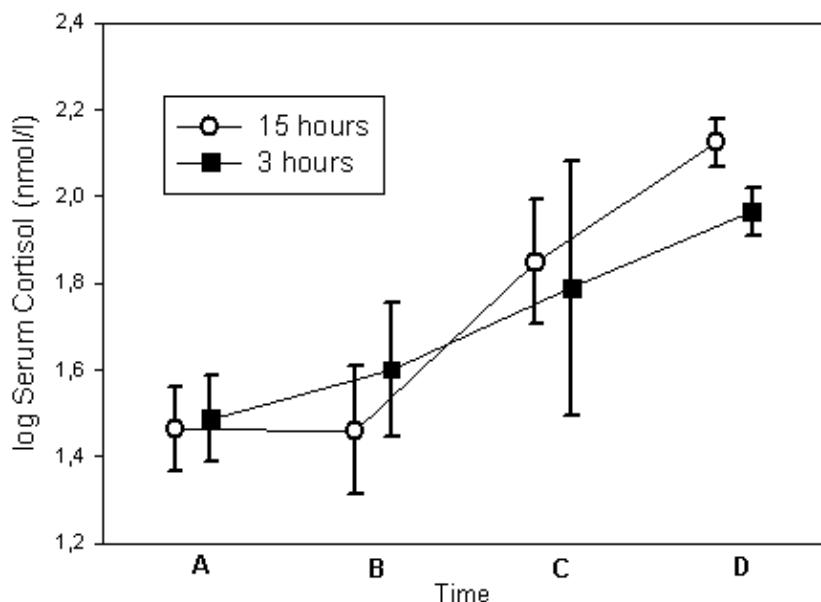


Figure 1. Serum cortisol (log) values at different times, within each slaughter group. Lines represent media and confidence interval.
A: basal value in farm; B: after transport; C: after lairage; D: at slaughter.

Lairage and preslaughter effect on cortisol

Serum cortisol concentrations significantly increased with respect to basal values after lairage and at slaughter. Cattle have very low basal levels, often less than 15 nmol/l (Boissy and Le Neindre, 1997; Lay et al., 1992) but in the present experiment they were around 30 nmol/l in both slaughter groups. Cortisol concentration in serum was no different after 3 and 15 hours in lairage ($p<0.05$). All animals were stressed, probably due to the inherent noises and movement of animals and people in the yards during routine handling and because of the new environment. It is known that after a stressful event, haematological variables can return to basal levels within 30 minutes if animals are in their familiar environment (Sartorelli et al., 1992). In this study, probably due to the new environment, higher values of cortisol were registered even after 15 hours in lairage.

Both groups also had a considerable preslaughter stress response, increasing to 90 and 130 nm/L in the short lairage and the overnight group, respectively (Figure 1). This is consistent with results from Boissy and Le Neindre (1997) and Lay et al (1992), who reported that cortisol levels in response to a stressor could increase up to 60-200 nmol/L in cattle. Some authors believe that the increase in cortisol concentrations during bleeding are mainly a response to handling in the race when driving the steers to the stunning box (Tadich et al., 2005). It is worth noting that they could also represent the cumulative effects of all stages of

the pre-slaughter handling. Moreover, due to food safety requirements, cattle are washed on their way to the stunning box to remove hide or fleece contaminants such as excreta and dirt. The process of handling and washing the animals would have elicited a stress response which could partially explain the cortisol rise in both slaughter groups. Although the distance between washing and stunning is short, it could have been enough to raise HPA axis activity. In addition, the effect of the process of stunning itself cannot be disregarded.

Calmer animals showed lower cortisol values in blood at slaughter, regardless of diet or slaughter group (see regression coefficients in Table 2; Figure 2; $p<0.05$).

Table 2. Effect of lairage time, Average Tindex and final live weight on serum cortisol concentration at slaughter.

	Parameter Estimator	Pr > t
Intercept	2.54328	<.005
Lairage time (Dummy)	-0.16518	<0.05
Average Tindex	-0.00176	<0.05
Final live weight	-0.00068	ns

*Lairage time (Dummy variables): 0= 15 hours; 1= 3 hours.

Calmer animals also showed lower cortisol concentrations in serum throughout the whole period (Estimator: -0.002, $p<0.05$). These results are consistent with those reported by Curley et al. (2008), who indicated that the functional characteristics of the HPA axis vary with animal temperament. Several authors reported elevated cortisol concentrations in cattle that are more agitated by human-animal interactions (i.e. exhibiting a faster EV) when compared to calmer animals (Curley et al., 2006; Fell et al., 1999). Our results seem to provide support to the recognized influence of temperament in modulating the adrenal response of cattle to different stressful situations.

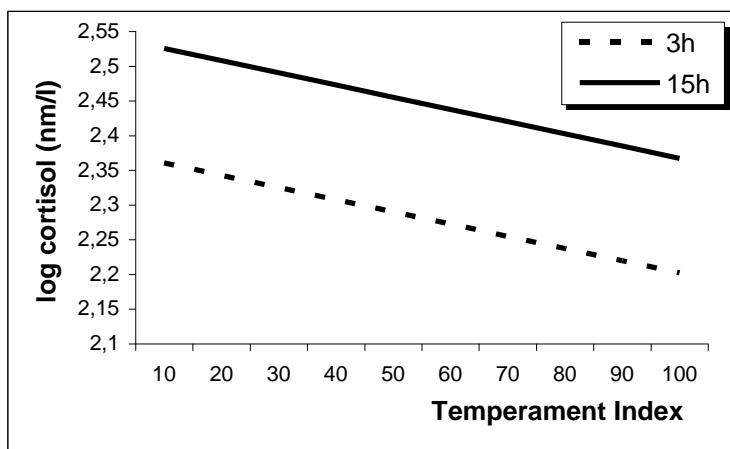


Figure 2. Average Tindex effect on cortisol (log) values at slaughter. Trendlines per slaughter group, estimated by regression analysis ($R^2=0.30$).

Transport effect on CPK and NEFA

Serum CPK concentrations significantly increased with respect to basal values after transportation in both slaughter groups (Table 3). Similar results were found by several other authors (Grasso et al., 1989; Groth and Granzer, 1977; Van de Water et al., 2003; Villaroel et al., 2003). Plasma creatine kinase is a muscle-specific enzyme whose activity in the blood is useful for indicating leakage from the muscle as a result of trauma, physical exercise or other damage (Lefebvre et al., 1996).

Table 3. CPK (log) values at different times within each slaughter group.
Least square means \pm Standard error.

Log CPK (U/L)	Basal value	After transportation	After lairage	At slaughter
3 hours	1.99 ^c \pm 0.50	2.48 ^b \pm 0.51	2.39 ^b \pm 0.52 b	2.71 ^a \pm 0.51
15 hours	2.08 ^c \pm 0.50	2.44 ^b \pm 0.51	2.40 ^b \pm 0.51 b	2.67 ^a \pm 0.50

Values with different letters differ $p < 0.05$

The increased activity of the enzyme in this experiment could indicate possible trauma during loading, transport and unloading, or it could have increased as a result of behavioural interactions between steers (Anderson et al., 1976; Lefebvre et al., 1996). Even if driving is smooth, animals need to make a considerable physical effort during transportation to keep their balance (stability) and posture. Vibration and motion might also have caused stress, so that, travelling could have been an accumulation of the non-specific stress response and the physical effort (absolute values in U/L increased 2 times after transport and 4 times at slaughter with respect to basal values in both groups).

NEFA concentrations also increased in both slaughter groups after transportation (Table 4). Similar results were found by Warriss et al. (1995), who reported that transport of cattle for between 5 and 15 h was associated with increases in blood concentrations of free fatty acids indicating that the cattle mobilised body energy reserves. Changes in these blood metabolites are indicative of energy mobilization, a mechanism necessary to maintain homeostasis (Moberg and Mench, 2000). Fasting and stressful events are typically associated with increased energy demands and this leads to depletion of energy stores, in particular liver glycogens and body fat (Balm, 1990). In the present experiment, the higher NEFA concentrations after transport are consistent with CPK increases reported before. As mentioned, free fatty acids may increase during feed restriction as a result of fat reserves being mobilised to supply energy requirements, but they may also increase in response to catecholamine release following acute stress (Shaw and Tume, 1992). Although cortisol concentrations did not increase after transport, it is possible that sudden moments of

extremely acute stress (like sudden truck movements or vibrations), provoked activation of the autonomic nervous system with the consequent increase in NEFA, although it was not enough to activate the HPA axis. According to Mellor and Stafford (1997) the relatively slow response time of the HPA axis may make it insensitive as a means of discriminating different level of stress within the first few minutes after a noxious stimulus. The physiological changes elicited by the sympathetic adrenomedullary system may be more accurate in assessing the early stages of distress response (Mellor et al., 2000). In our experiment, physical stress was evident after transport, according to CPK and NEFA concentrations, but results showed that the situation did not involve the HPA axis activity. The activation of the HPA-axis is mainly dependent on the emotional involvement of the animal; stressors do not necessarily activate the HPA system when the animal does not perceive the situation as stressful (von Borell, 2001).

We could not conclude from the results obtained that animals were suffering during transport. The physiological changes registered in this stage seem to be an indication that the adaptive mechanisms were functioning.

Lairage and preslaughter effect on CPK and NEFA

After lairage, CPK values did not increase in any slaughter group (Table 3). Similar results were found by Tadich et al. (2005) who found higher CPK activity after transport (with 0, 3 and 16 hours) but did not find an additional increase during lairage (in different combinations of transport: 0, 3, 16 hours; and lairage duration: 0, 3, 12, 16 and 24 hours). In our experiment, negative behaviour during lairage (mounting and fighting) were more frequent in the 3-hour group (see behavioural analysis), but this higher activity/exercise was apparently not enough to increase serum CPK concentrations. NEFA concentrations were higher after lairage but only in animals from the overnight group (Table 4), suggesting a greater energy demand to restore homeostasis because of the longer food deprivation (Gupta et al., 2005). These differences could therefore be explained as a result of fat reserves being mobilised to supply energy requirements, probably with the additional effect of psychological stress due to the new environment. However, as has been mentioned, HPA axis activity increased but did not differ between groups during lairage.

Here again, physiological results did not allow us to conclude that there was a higher degree of suffering in the long lairage group.

Preslaughter handling procedures had a significant effect on CPK values in both groups (Table 3). The higher presence of CPK implies constant muscle movement, both voluntary and those that are controlled by the autonomic nervous system (heart, lungs). Elevated plasma CPK activity is also associated with strenuous or unaccustomed muscular exercise

(Berg and Haralambie, 1978). For this reason we considered that the stunning process itself could have had a considerable effect on these results (tonic and clonic phases).

Calmer animals had lower CPK and NEFA values in serum throughout the experiment ($p<0.05$). The effect of temperament on the stress response in cattle has been already discussed in this paper.

Table 4. NEFA values at different times within each slaughter group.
Least square means \pm Standard error.

NEFA (mmol/L)	Basal value	After transportation	After lairage	At slaughter
3 hours	0.36 ^d \pm 0.02	0.55 ^b \pm 0.03	0.48 ^{bcd} \pm 0.08	0.43 ^{cd} \pm 0.03
15 hours	0.37 ^d \pm 0.02	0.49 ^{bc} \pm 0.03	0.68 ^a \pm 0.04	0.52 ^b \pm 0.03

Values with different letters differ $p<0.05$

Cattle kept in lairage overnight had greater NEFA concentration at slaughter than the 3-hour group, whose values remained unaltered after lairage and were no different from basal concentrations (Table 4). The short lairage group was only affected after transportation, suggesting that food deprivation was not long enough to cause a lasting rise in NEFA. Increases after transport could probably have been related to links with the adrenomedullary system activity. Although NEFA slaughter values in the long lairage group were lower than in the post-lairage, recovery was not enough to achieve basal values. Undoubtedly, overnight animals presented higher energy demands than the 3 hour-group. These results are consistent with those from Jarvis et al (1996) who reported higher concentrations of NEFA during bleeding ($p<0.05$) in animals that spent more than 16 hours in the abattoir (overnight) when compared to animals that spent 5 hours in lairage pens previous to slaughter (0.28 and 0.33 mmol/l respectively). Cockram and Corley (1991) also found that cattle held overnight in lairage had significantly greater plasma-free fatty-acid concentrations than those slaughtered on the day of arrival. However, because of the experimental design, those authors could not separate the specific lairage effect from the preslaughter handling effect. In our experiment, NEFA differences between both groups were established after lairage.

β -hidroxibutirate (β HB) serum concentrations were higher ($p<0.05$) in animals from D2 (0.32 ± 0.02 vs 0.28 ± 0.01 in D1) with no differences between slaughter groups. Ketonic bodies, like β HB are excellent fuel for tissue respiration, in particular when glucose levels are limited (fastening). However, under these circumstances, these tissues can easily use NEFA energy sources. In the present study probably fasting was not long enough to cause a strong and clear β HB stress response or to determine differences between slaughter groups.

Lairage pen behaviour

The animals did not drink water during lairage. It is possible that this behaviour was suppressed as a result of unfamiliarity with the new environment. However, hematocrit values at slaughter showed that animals were not dehydrated. If cattle are fully hydrated and fed before transport, it is likely that food deprivation rather than water, will be the greater stressor over the initial 24 hours, since this is more likely to disrupt rumen function (Hartung et al., 2000). From practical experience in our particular abattoir, we have seen that animals do not drink water while in lairage pens. In the present experiment, animals did not perform social behaviour, self grooming or lie down during the scan. The time budget is shown in Figure 3.

According to the scan sampling technique, steers from both slaughter groups spent around 80% of total time in lairage, standing/ruminating ($p<0.05$; Figure 3) and no differences were registered in walking and negative behaviour. Results from the Glimmix procedure showed that animals from D2 spent more time ruminating than supplemented steers (D1) ($p< 0.05$). Animals are known to ruminate while resting (Tribe, 1955) so these results suggested that animals from D2 were probably calmer than those from D1. Temperament and breed did not have any affect on the time budget.

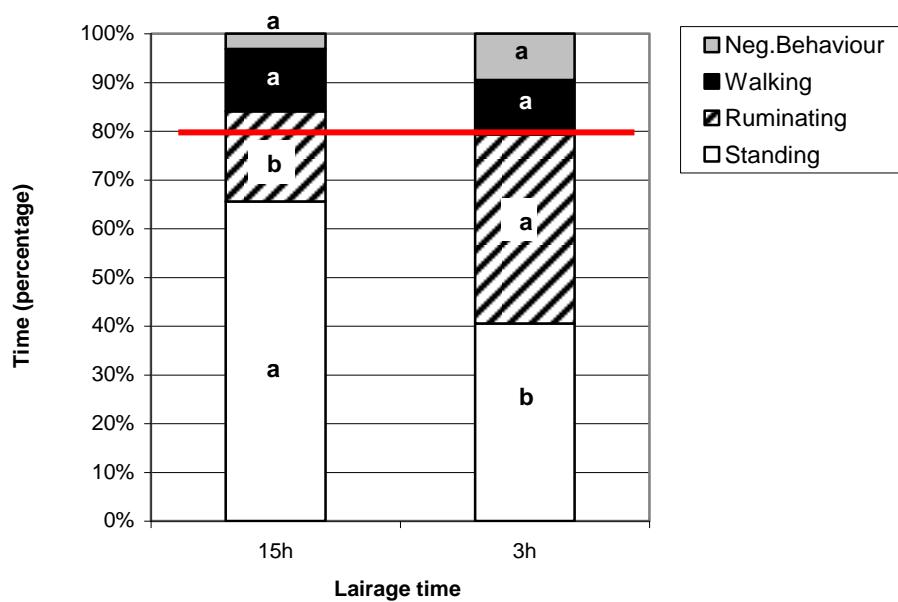


Figure 3. Percentage of the total time spent on each of the observed behaviour, by slaughter group.

Note: The same activity with different letter (between bars) differs with $p < 0.05$.

Figure 4 describes each behaviour evolution in the long lairage group, suggesting that the first hour was the most critical, with less rumination and more walking and negative behaviour.

As mentioned, negative behaviour would have been missed by scan sampling (Martin and Bateson, 1993). In fact, according to the *Behaviour sampling technique*, fighting frequency (fights/hour) was significantly higher in the short lairage group ($p<0.05$) and for Braford steers ($p<0.05$). Results from the Glimmix procedure also showed that supplemented steers (D1) were more aggressive than those from D2, and these results were consistent with the higher rumination time registered in animals from D2.

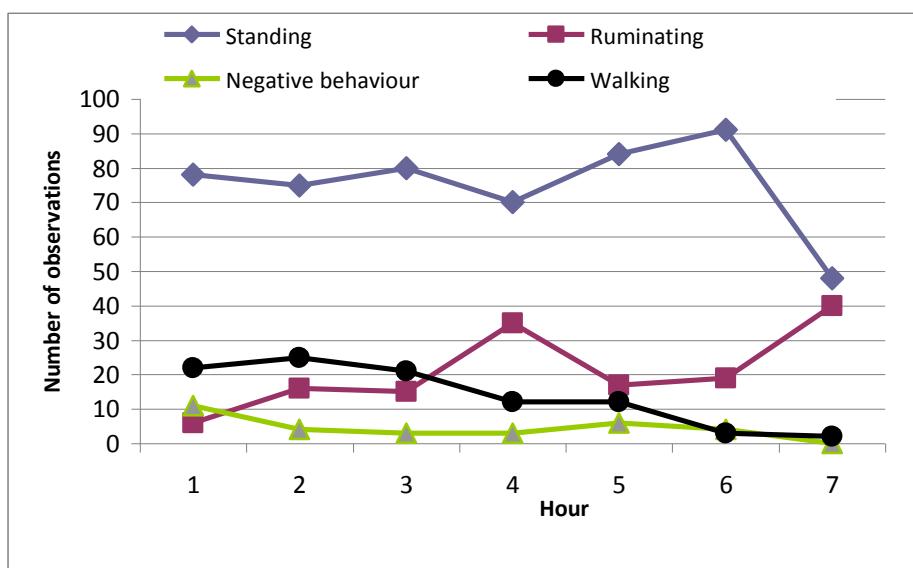


Figure 4. Frequency of different behaviours at each hour of lairage (long lairage group).

When analyzing fighting frequency during the first hour in lairage, no differences were found between slaughter groups (Figure 5; $p<0.05$). The frequency of this activity in consecutive hours (obviously in the long lairage group) was therefore compared to fighting frequency during the first hour. Results from each Binomial proportion comparison showed that fighting frequency in the first hour in pens was significantly higher than the second, third, forth, fifth, sixth and seventh hour, respectively (Figure 5; $p<0.05$). Based on these results, we could infer that the first hour in pens was a critical adaptation stage for both groups. The animals that remained in pens became calmer afterwards. According to these results we could have expected the same evolution in fighting frequency in the short lairage group. The lowest fighting frequency in the 15 hours group (with respect to the first hour) was registered during the 4th and 7th hour (Figure 5, $p<0.05$). Temperament and breed did not have an effect on fighting frequency during the first hour in lairage, suggesting that it was a stressful period for all animals.

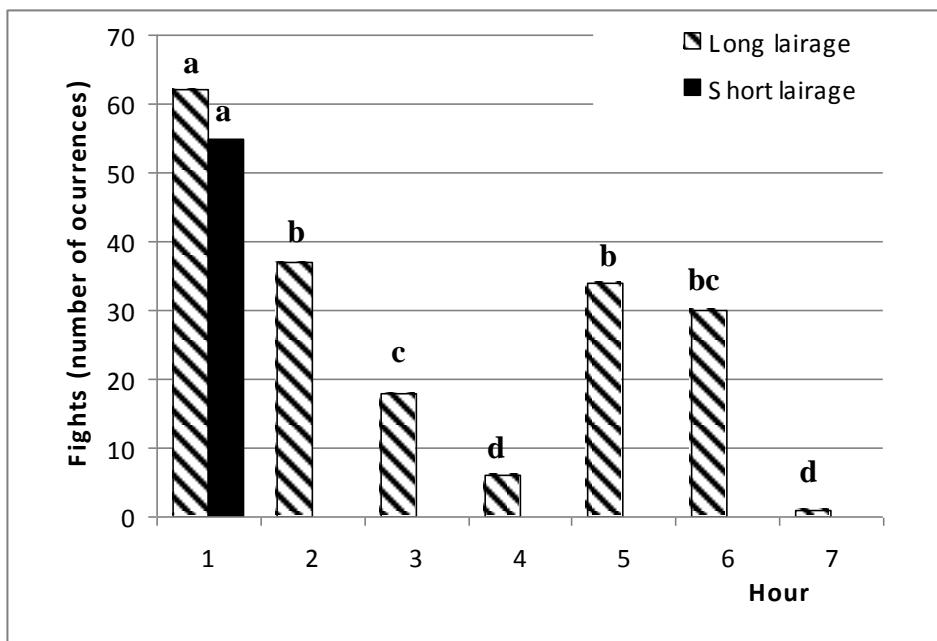


Figure 5. Number of fights during consecutive hours for each slaughter group.
Bars with different letter differ $p<0.05$

Both groups were situated in quiet environments far from the unloading facilities, but the 15-hour group waited overnight, with greater opportunities to rest. Noise generated by the normal abattoir activity was noticeably higher during the morning and mid-day because of the slaughter procedures. This could have contributed to a higher excitability in the 3-hour group, not having the opportunity to rest or to get used to the pens. However, considering that there were no differences between groups in fighting frequency during the first hour of observation, we consider that lack of resting time was probably the most important reason for these results. "Fights" could have been an adaptive mechanism for all steers, but even if they were showing adaptive behaviour, they may still be suffering in the process. Conflict may be beneficial in the long run, but will still be unpleasant while it lasts (Dawkins, 1980), especially considering those animals that did not have enough time to cope with the new situation (3-hour group). Based strictly on physiological results from times A to D, we could have concluded that animals from the overnight group were physically and emotionally more stressed than the 3-hour group. However, according to behaviour results, the short lairage group was more excited during lairage and carcasses showed higher pH values.

3.3. Carcass traits

pH

Carcasses from the short lairage group had higher values of final pH (5.83 ± 0.04 vs 5.68 ± 0.04 in the long lairage group; $p<0.05$). It seemed that their excitability without having the opportunity to recover, implied a significant depletion of muscle glycogen reserves with a

profound effect on pH at 24 hours *post mortem*. There was thus, direct evidence that fighting activity during lairage resulted in a higher last muscle pH, as found by Grigor et al. (2004) and Warris et al. (1984). Stressors appear to be additive (Bray et al., 1989) so that multiple stressors without the opportunity to recover during lairage resulted in a greater elevation of muscle pH.

Bruising

Incidence of bruising was not significantly affected by lairage time, with 14 bruises registered in the overnight group and 15 in the short lairage group. These results are not consistent with those of Mc.Nally and Warris (1996) who reported higher bruise incidence in carcasses from cattle that remained for longer lairage periods. It is important to consider that bruise incidence in this study was registered according to welfare criteria. This means that minor bruises implied subcutaneous tissue remotion only, or any deterioration in the region affected. In spite of the relatively low incidence of bruising, we consider that the bleeding procedures (pre and post lairage) undoubtedly contributed to the presence of minor and especially major bruises. Major bruises affecting carcass and meat quality were only registered in the overnight group (1 bruise in two animals) and both steers had jumped through the chute while being bled. According to some authors, the vigorous avoidance response of cattle with poor temperament in confined areas during handling, transport and pre-slaughter increases the likelihood of falling and of collision with yard or stock crate structures, and also with other cattle, increasing the chance of bruising (Barnett et al., 1984). In the present study, results from a logistic regression analysis showed that temperament was not related to bruise incidence ($p<0.05$). The good management practices followed in the abattoir could have contributed to these results.

4. Conclusions

Considering average daily gains, environmental conditions, animal health performance and mortality rate, it is possible to make the preliminary inference that animal welfare was not compromised in any diet during the finishing period. Frequent and proper handling was considered to be very important, especially when working with excitable animals. Temperament appears to be an important feature with regard to its effect on productivity (ADG) and also on the individual stress response at different pre-slaughter stages. Transportation is, in general, inevitably associated with a stress response but its negative effects may be avoided or minimised after short travels (less than 4 hours) by proper handling and the use of suitable equipment and facilities. Increases in energy demands are unavoidable in fasting animals, especially with longer lairage, but adequate conditions and a calm environment may allow cattle to rest and recover while waiting in pens, with positive

effects on pH values. Lairage and preslaughter handling induced a significant increase in the activity of the hypothalamus-hypophysis-adrenal axis, suggesting some degree of psychological stress. The first hour in lairage was a stressful period and animals became calmer afterwards. The insufficient resting period remained animals highly excitable before slaughter, contributing to glycogen depletion and to the consequent higher pH values registered. The results of this experiment suggest that more than 3 hours of resting in lairage appears to be desirable from a welfare point of view. We consider that an intermediate lairage duration would also be appropriate from the meat-quality perspective. Nevertheless, our data do not enable us to recommend a specific waiting time and further research is needed to clarify this issue.

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CAPÍTULO VIII - DISCUSIÓN GENERAL

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Las alternativas de alimentación evaluadas en ambos experimentos permitieron ganancias de peso adecuadas a los objetivos planteados, constituyendo opciones viables para la terminación de novillos en diferentes épocas del año, con diferente nivel de intensificación. En general, las ganancias de peso de los animales estuvieron directamente relacionadas al nivel de energía de la dieta, determinando diferentes fechas de terminación y faena. Si bien desde el punto de vista productivo la mejor alternativa fue la terminación a corral, dichos animales vieron afectado su bienestar a nivel individual, tanto en lo que se refiere a una mayor incidencia de enfermedades, así como a una mayor tasa de mortalidad. La salud física constituye una condición fundamental para un adecuado bienestar, sin embargo, el concepto de salud es más amplio que la simple ausencia de enfermedad. En este sentido, se destaca que los animales confinados mostraron una mayor dificultad para habituarse a las condiciones de producción, según indicadores fisiológicos relativos al estrés. Dichas dificultades podrían atribuirse en parte a la privación de realizar comportamientos probablemente relevantes para el animal (como el pastoreo y la exploración), al mayor hacinamiento y/o debido a su menor capacidad de respuesta frente a inclemencias climáticas. Se deberían estudiar y entender los sistemas motivacionales o bases fisiológicas que existen detrás de cada comportamiento individual, de forma de poder concluir acerca del efecto de su privación sobre el Bienestar animal. Las estereotipias en bovinos no son tan comunes como en otras especies. Sin embargo, su evaluación, junto a la ocurrencia de comportamientos negativos en la fase de producción, podría constituir una herramienta adecuada para la determinación de posibles estados de frustración, aburrimiento u otro tipo de sufrimiento, especialmente en animales confinados.

Respecto al comportamiento de los animales alimentados en base a pastura, los resultados de este trabajo sugieren que la inclusión de grano en la dieta puede cambiar la proporción relativa de tiempo destinada a las diferentes conductas de pastoreo, dependiendo de la calidad y la cantidad de la dieta (tanto de la pastura como del suplemento). Dichos cambios no afectarían el bienestar de los animales, siempre que no se vean afectados los patrones diarios de comportamiento en pastoreo y se tomen estrictas medidas de prevención de enfermedades derivadas de la dieta.

Los animales de temperamento más calmo presentaron mayores ganancias de peso en todas las alternativas evaluadas y en las diferentes razas. El cumplimiento de protocolos de buenas prácticas de manejo presentó un efecto positivo en la evolución del temperamento de los animales Hereford, especialmente en aquellos sistemas de producción que implicaron mayor contacto con el hombre (suplementados). Los animales de raza Braford fueron más

excitables, temperamentales y difíciles de manejar que los de raza Hereford en las condiciones de producción evaluadas. A pesar de que estas diferencias podrían deberse en parte a factores ambientales, las diferencias genéticas en cuanto a docilidad o excitabilidad del ganado bovino han sido demostradas por diferentes autores y discutidas en el presente trabajo. Si bien no se registraron eventos importantes de agresividad durante los experimentos, se destaca que el temperamento de los novillos Braford no evolucionó en forma positiva ni siquiera en el tratamiento suplementado. Esto implicaría que el cumplimiento de buenas prácticas de manejo se vuelve aún más relevante al momento de trabajar con razas más excitables.

Se considera que se deberían estandarizar tests para la medición del temperamento en bovinos. Una herramienta para medir temperamento debe ser confiable, repetible y deberá estar vinculada a la respuesta individual frente al estrés. Diversos estudios han utilizado diferentes técnicas siendo muchas de ellas subjetivas, lo cual incrementaría las posibilidades de que exista error humano y/o sesgo en la determinación del temperamento de los animales. El "Flight Time" se considera una herramienta objetiva, segura y rápida que puede ser implementada fácilmente a nivel de producción. Si bien en el presente trabajo se utilizó un índice de temperamento que combinaba resultados de test objetivos y subjetivos (habiéndose comprobado la correlación entre dichas medidas), se considera que el Flight Time por sí solo, sería una herramienta suficiente.

En los últimos años se ha avanzado mucho en el entendimiento de las relaciones del hombre con los animales, demostrando la gran influencia que presenta el ser humano en lo que tiene que ver con la productividad y el bienestar de los animales domésticos en producción. Al respecto, se considera que la comunidad en general debe dar un énfasis incremental al aseguramiento de la capacitación de los operarios rurales y la gente que trabaja con animales en producción. Más allá del sistema de producción, uno de los factores más importantes y por tanto uno de nuestros mayores desafíos como país productor de carne en sistemas extensivos, sería la capacitación del personal que trabaja con los animales. Las buenas prácticas de manejo se verán reflejadas tanto en un incremento de producción como en la mejora de la calidad del producto obtenido, lo que puede traducirse en efectos positivos sobre los ingresos y la rentabilidad de las empresas agropecuarias. Se considera que los retailers o mayoristas podrían jugar un rol fundamental a la hora de la valoración y cumplimiento de normas relativas al Bienestar animal. Estos podrían imponer y exigir a sus abastecedores, el cumplimiento de Códigos de Buenas Prácticas de Manejo, especificando estándares de BA que abarquen todos los aspectos de la producción de carne, desde el nacimiento del animal hasta el momento de la faena.

En la práctica actual, el ayuno previo a la faena en el Uruguay es de alrededor de 12-15 horas y se realiza habitualmente para disminuir el contenido gastrointestinal y así reducir el riesgo de contaminación de las canales al momento del eviscerado, para dar tiempo a la inspección veterinaria en pie y además para permitir la planificación de la faena. Al evaluar dos tiempos contrastantes de espera previo a la faena (3 y 15 horas), no se registró un efecto del sistema de alimentación sobre la respuesta al estrés en las diferentes etapas evaluadas (transporte, espera en corrales y traslado al cajón de noqueo). Se destaca que animales de temperamento más calmo tuvieron una menor respuesta fisiológica en las distintas etapas evaluadas, independientemente del tratamiento y del tiempo de espera.

En general, se considera que el estrés psicológico y físico sería inevitable durante las etapas previas a la faena. Sin embargo, los resultados de este trabajo sugieren que la respuesta psicológica frente al estrés del transporte podría reducirse e incluso minimizarse, a través del cumplimiento de adecuadas medidas de manejo (buenas condiciones del camión y calidad de la conducción, rutas en buen estado, respeto de la carga recomendada, manejo correcto durante la carga y la descarga, otros). En el presente trabajo, los animales presentaron indicios de estrés físico luego de la espera y este fue mayor en la espera de 15 horas. Sin embargo, al integrar diversos indicadores asociados al BA (conducta en corrales, frecuencia de comportamientos negativos, descenso de pH en la canal, terneza de la carne), no fue posible deducir que dicha respuesta provocara un deterioro del bienestar de los animales pertenecientes a ese grupo. El hecho de haberles otorgado buenas condiciones de espera y un ambiente calmo, permitió que los animales de 15 horas se recuperaran físicamente, logrando adecuados descensos de pH y consecuentemente menores valores de fuerza de corte en la carne. Los animales que permanecieron un período corto en corrales, presentaron altos valores de pH final y una mayor dureza de la carne. Esto en parte podría ser explicado por la alta excitabilidad, habiéndose registrado una frecuencia elevada de comportamientos negativos durante la primera hora de observación. Es importante considerar que si bien esto ocurrió en ambos grupos de faena, los animales de la espera corta no tuvieron suficiente tiempo ni posiblemente un ambiente lo suficientemente calmo como para descansar y recuperarse antes del sacrificio.

El momento inmediatamente previo al noqueo parece ser de gran relevancia en lo que tiene que ver con respuestas fisiológicas de estrés. En el experimento 1 se observó un incremento significativo de las proteínas de fase aguda en dicho momento y en el Experimento 2 los animales sufrieron un importante incremento de los niveles de corticosteroides en sangre en el momento previo al noqueo, sugiriendo un estado de estrés emocional considerable que deberá estudiarse con mayor profundidad.

El temperamento parece constituir una herramienta fundamental para reducir el estrés tanto físico como emocional en las diferentes etapas pre faena (transporte por carretera, espera en corrales, traslado al cajón de noqueo). Los factores estresantes parecen ser aditivos, por lo que la ocurrencia de factores estresantes múltiples en las etapas previo a la faena, tendrían un efecto mayor sobre el Bienestar animal y la calidad de la carne que cuando ocurren en forma aislada, siendo aún más importante este efecto en animales más excitables.

De acuerdo a los resultados del experimento 2, se considera que 3 horas de espera en corrales previo a la faena no serían suficientes desde el punto de Bienestar animal y de la calidad de la carne. Se deberán evaluar alternativas intermedias de duración de la espera, de forma de combinar ambos criterios.

Los tratamientos en base a pastura con la utilización de grano como suplemento, mostraron los mayores valores de peso de canal caliente y peso de cortes valiosos en ambos experimentos, incluso más que en el tratamiento a corral del experimento 1. Sin embargo, en ninguna de las alternativas evaluadas se detectaron diferencias en el rendimiento de carne. No se obtuvieron diferencias en proporción de músculo en el corte pistola con las diferentes alternativas de intensificación, pero el porcentaje de grasa de la misma fue mayor en los tratamientos con mayor nivel de grano en la dieta en ambos experimentos.

En el experimento 2 que incluyó animales de raza Braford, se observó que dichos animales presentaron mayor proporción de músculo en la pistola que los Hereford, mayor peso de la pistola, de los cortes valiosos y del R&L y también mayor rendimiento de los cortes valiosos, independientemente del sistema de alimentación. Al compararlos al mismo peso vivo, los animales de razas de maduración tardía tal como Braford, son más jóvenes y se encuentran aún deponiendo proteína mientras que los de maduración temprana o precoces como Hereford, ya alcanzaron su límite fisiológico y comenzaron la fase de deposición de grasa. Desde el punto de vista económico los resultados son muy interesantes, pero debe considerarse que los animales Braford tuvieron mayores valores de fuerza de corte de la carne. Desde el punto de vista científico, se comprueba que es importante la comparación de animales de diferente raza, a una misma edad fisiológica (punto de madurez fisiológica).

En el experimento 1 los animales de pastura presentaron grasas con un mayor índice de amarillo, disminuyendo conforme aumentaron los niveles de grano en la dieta. Sin embargo, en el Experimento 2 no se encontraron diferencias en el color de la grasa. Los valores de b^* de los tratamientos del Experimento 2 ($b^*= 19$ en el tratamiento suplementado y $b^*= 18$ en el mejoramiento de campo) fueron similares a los obtenidos en el Tratamiento 1 ($b^*= 19$) y el Tratamiento 2 ($b^*= 18$) del Experimento 1. Se considera que el color de la grasa de

animales alimentados en base a pastura podría mejorarse con niveles de grano en la dieta mayores al 1 % del peso vivo. De hecho, en el Experimento 1 dichas diferencias fueron consideradas relevantes a partir de la inclusión de grano a niveles de 1.2% del peso vivo. De todas formas, se considera que desde el punto de vista de la percepción del consumidor, el color de la grasa no constituiría un factor negativo en la carne de animales provenientes de pastura, dado el “dressing” o nivel de remoción de grasa subcutánea que requieren los mercados de exportación.

Respecto al color de la carne, en el Experimento 1 no se registraron diferencias entre los tratamientos en base a pasturas (T1, T2 y T3), pero todos presentaron carnes con mayor índice de rojo que la proveniente del sistema a corral. En el tratamiento 2 tampoco se registraron diferencias en el color de la carne atribuidas a la dieta ni entre las diferentes razas. Se considera que los procesos de transformación que ocurren en el rumen podrían ser una de las causas por las que generalmente no se encuentran diferencias debidas a la dieta, en el color de la carne. Sin embargo, la carne de animales que estuvieron más tiempo en corrales de espera presentó un índice de rojo mayor que la del grupo de espera corta. Se considera que dichas diferencias de color fueron debidas a la relación inversa que existe entre el pH final y el color de la carne, habiéndose alcanzado menores valores de pH último en el grupo que permaneció 15 horas en corrales.

La tasa de descenso de pH determinó menores valores de fuerza de corte en los animales que permanecieron durante toda la noche en corrales de espera. El descanso podría haber permitido que los animales repusieran los niveles de glucógeno del músculo con el consecuente efecto positivo tanto sobre el color como sobre la terneza de la carne. Los animales del grupo de 3 horas, probablemente debido al mayor estrés y a la imposibilidad de descansar y recuperarse mientras permanecían en corrales, no tuvieron niveles de glucógeno suficientes como para permitir una correcta acidificación del músculo *post mortem*. Los altos valores de pH a la hora, a las 3 y a las 6 horas, incluso cuando la temperatura descendió a 12 grados, confirmarían dicha hipótesis.

De todas formas, se destaca que los valores de fuerza de corte de este experimento fueron mayores a los registrados en el Experimento 1. Dentro del Experimento 2, los valores de fuerza de corte registrados en animales de la raza Braford fueron mayores a los de la raza Hereford. La carne de las razas índicas y continentales es menos tierna que la carne de razas de origen británico, independientemente del ambiente en el cual el animal produce. Algunos autores atribuyen estas diferencias a un posible mayor engrasamiento de las razas británicas, lo cual no fue el determinante en este caso, no habiéndose registrado diferencias en el nivel de engrasamiento entre ambas razas (% de grasa en la pistola y espesor de grasa subcutánea). Si bien en el presente experimento no se midió el nivel de proteólisis ni

las características del colágeno, se considera que las diferencias en la fuerza de corte entre las distintas razas podrían estar explicadas por el resultado de una menor proteólisis (acción de la calpastatina) o por un efecto significativo de la presencia de sangre indica en el contenido total e insoluble de colágeno.

Es interesante destacar que en el Experimento 1 la carne de los animales provenientes de pastura presentó menores valores de fuerza de corte que la carne de feed lot. Existe información contradictoria al respecto, pero en general la bibliografía sostiene que la terneza es mayor en la carne proveniente de sistemas de alimentación que incluyen un mayor nivel de grano o concentrado en la dieta. Las mayores ganancias diarias logradas en dichos sistemas podrían provocar una mayor degradación posterior de las proteínas. A su vez, las mayores ganancias diarias logradas con mayores valores energéticos de la dieta, permitirían alcanzar el punto de faena con animales más jóvenes y por tanto con menor contenido de colágeno así como con una mayor solubilidad del mismo. Más allá de dichos argumentos, en el presente experimento los animales de pasturas tuvieron menores ganancias durante el período de engorde y por tanto alcanzaron el punto de faena con una edad mayor que los novillos del feed lot. Sin embargo, la carne de animales provenientes de pastura presentó valores de fuerza de corte significativamente menores a la de los animales de feed lot. Se considera que el patrón de descenso conjunto de pH y temperatura especialmente en las primeras horas *post mortem*, probablemente haya hecho que el ambiente fuera más favorable para la acción de las enzimas proteolíticas en el tratamiento de pasturas.

La carne proveniente de animales más calmos, presentó menores valores de fuerza de corte en ambos Experimentos, independientemente del sistema de alimentación y de la raza. Este efecto del temperamento sobre la terneza podría deberse a la acción que presenta la unión de la adrenalina con los receptores adrenérgicos (a nivel de la membrana celular muscular), sobre el metabolismo del glucógeno del músculo *in vivo*. Animales más temperamentales serían más susceptibles ante situaciones de estrés, especialmente en las etapas previas al sacrificio. La mayor descarga simpática provocaría el consumo del glucógeno del músculo impidiendo la correcta acidificación del mismo y afectando en forma negativa las características organolépticas de la carne. Las catecolaminas (adrenalina), podrían además inhibir el sistema proteolítico en el proceso de transformación del músculo en carne, especialmente el sistema de las calpaínas, encargado de la proteólisis en las fases *post mortem* tempranas.

Por otra parte, podría existir un efecto negativo del estrés y por tanto del temperamento sobre la terneza, a través de la acción de ciertas proteínas que se encargan de prevenir la apoptosis o muerte celular. Dichas proteínas son producidas por las células del animal como

forma de defensa cuando éste se enfrenta a una situación de estrés. Por lo tanto, en el período inmediato a la muerte, éstas podrían enlentecer el proceso de muerte celular constituyendo un obstáculo para la maduración.

Según los resultados de estos trabajos, deberían analizarse los cambios bioquímicos y estructurales que ocurren luego de la muerte del animal, en forma previa a las fases de *rigor mortis* y maduración.

El conocimiento científico ha logrado grandes avances en lo que tiene que ver con la bioquímica del músculo y los eventos metabólicos que ocurren luego del sacrificio. Sin embargo, no debe despreciarse la identificación de herramientas o factores a nivel de sistemas productivos que contribuyan al logro de un producto de buena calidad. En este sentido, la consideración del temperamento animal parece ser una herramienta válida. Los resultados de este trabajo sugieren que el temperamento podría presentar una influencia sustancial sobre la rentabilidad de las empresas de producción de carne. Dicho efecto podría manifestarse tanto a través de la relación temperamento-crecimiento o engorde, así como a través de la relación temperamento-calidad de canal y carne.

CAPÍTULO IX - CONCLUSIONES Y DESAFÍOS DE LA INVESTIGACIÓN

CAPÍTULO IX - CONCLUSIONES Y DESAFÍOS DE LA INVESTIGACIÓN

A partir de ambos experimentos es posible concluir que independientemente del sistema de producción y la raza utilizada, el temperamento de los animales constituiría una herramienta muy importante tanto desde el punto de vista productivo, de la respuesta individual ante diferentes situaciones estresantes previas a la faena, así como de la calidad de la carne.

La inclusión de niveles incrementales de grano en la dieta hasta 1.2 % del peso vivo, permitiría mejorar la performance animal sin afectar el Bienestar animal, siempre que se tomen medidas preventivas estrictas respecto a enfermedades provocadas por la dieta.

La estrategia de terminación a corral, si bien incrementa los niveles de producción, comprometería el bienestar de los animales y la calidad de la carne producida.

El cumplimiento de buenas prácticas de manejo se considera de gran relevancia a los efectos del BA y la calidad de la carne, tanto en la fase de producción como en las diferentes etapas previas a la faena.

El estrés manifestado por los animales en la etapa inmediatamente anterior al sacrificio es de fundamental importancia y ameritaría futuros estudios específicos.

La calidad de la carne de animales terminados estrictamente en base a pastura no fue menor a la de aquellos sistemas que incluyeron diferentes niveles de grano en la dieta, presentando incluso menores valores de fuerza de corte que la carne de animales provenientes del sistema confinado.

La utilización de animales Braford podría incrementar el rendimiento de carne pero se deben considerar sus desventajas en cuanto al temperamento de los individuos y los mayores valores de fuerza de corte de la carne.

Respecto al tiempo de espera en corrales previo a la faena, se considera que se debe otorgar un tiempo prudente a los animales (mayor a 3 y posiblemente menor a 15 horas), que les permita descansar y eventualmente recuperar los niveles de glucógeno del músculo, de forma de lograr una mejor calidad del producto combinado a un adecuado Bienestar animal.

La Investigación en Bienestar animal: el caso de Uruguay

Nuestro desafío como investigadores en el área de producción animal es entender a los animales y su interacción con el ambiente de una manera tal, que haga posible una mayor producción de alimentos y de fibra, ocasionando el mínimo discomfort al animal y con un importante retorno económico para el productor. Para ello, se deberá comprender el comportamiento, las bases motivacionales de los mismos y la fisiología, a todos los niveles organizacionales, desde los patrones espaciales y sociales en un grupo de animales a pastoreo, hasta la acción de los neurotransmisores envueltos en procesos sinápticos en zonas específicas del cerebro.

La continuidad en los trabajos de investigación que incluyan comportamiento, indicadores fisiológicos de Bienestar animal, métodos para minimizar el estrés y las interacciones entre el medio ambiente, la sanidad y la productividad, serán el camino hacia la generación de conocimientos de sólida base científica para el cuidado y manejo adecuado de los animales en producción.

La Investigación en Bienestar animal deberá reflejar la realidad de los sectores primarios y agroindustriales ligados a las cadenas productivas involucradas, el estado del conocimiento científico y tecnológico, así como la opinión de los principales agentes del sector y de la sociedad.

La diversidad de enfoques, objetivos y metodologías utilizadas en los procesos de investigación a nivel internacional, demuestran que los hallazgos científicos no puedan ser aplicados a todos los contextos.

Se considera que la Investigación en BA en países como Uruguay con una arraigada cultura ganadera, no debe dejar de considerar la realidad social en la que se desarrolla la actividad agropecuaria. Es así que además del estudio y caracterización del BA en nuevas alternativas de producción, se deberán evaluar, cuantificar y comparar aquellas prácticas tradicionales que se realizan en el país, las cuales están cargadas de connotaciones culturales, pero que probablemente ameriten ser revisadas y/o mejoradas.

Por otra parte, es fundamental la participación y cooperación de todas las partes interesadas en la utilización de la información tecnológica generada, para el posterior diseño e implementación de normas de Bienestar animal que estén acordes a la realidad social y cultural, a los sistemas de producción y a las especies involucradas.

Este enfoque permitiría una adecuada respuesta ante la demanda, promover la adopción de los resultados obtenidos, una mejor situación estratégica frente a la imposición de barreras

no arancelarias, facilitar la cooperación tecnológica (nacional e internacional) y una mejora de la credibilidad (nacional e internacional).

ANEXO



Effects of feeding strategies including different proportion of pasture and concentrate, on carcass and meat quality traits in Uruguayan steers

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ABSTRACT

Eighty four steers were randomly assigned to three pasture treatments with increasing levels of grain (T1: 0%; T2: 0.6%; T3: 1.2% of live weight) and to an *ad libitum* concentrate treatment, T4, to study the effects on carcass and meat quality. Animals were slaughtered with 500 kg of average live weight per treatment. Average daily gain increased with increasing levels of energy, determining different slaughter dates. Intermediate treatments showed higher carcass weight than T1. T4 and T3 had a higher weight of valuable cuts than T1 and T4. Pistolas from T4 had a higher fat proportion and lower bone percentage. Increasing levels of energy in diet decreased fat yellowness. After 20 days of aging, T4 had the lowest muscle *a'* values and shear force was higher for T4 than for T1. With pastures finishing strategy, no adverse effects on meat quality were detected and tenderness was enhanced.

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1. Introduction

Uruguay is a primary meat exporter, being the 7th country in the world in volume, on which its economy is highly dependent. Meat production systems are mainly based on unimproved pastures but the use of intensive systems is growing in order to improve animal performance and carcass and meat quality traits, as well as to increase the number of animals in the system. These intensive systems include a wide range of feeding alternatives between pasture and concentrate utilization. The use of concentrates in pastures during limited periods or for finishing purposes is becoming more common. This could involve many differences in terms of carcass and meat quality, which require to be studied. The challenge is to produce a finishing strategy to improve the product without modifying the peculiar characteristics acquired during extensive grazing conditions (low-cost production and healthy meat for human consumption), and without compromising neither animal welfare status nor the environment. Finishing strategies in cattle have been extensively studied over the years with varied results on carcass traits and meat quality. Many studies have traditionally discounted meat tenderness from extensive systems as compared to concentrate-fed beef (Christall, 1994) and also colour (French et al., 2000). In many of these experiments, dietary effects were confused with animal age, pre-slaughter

growth rate or carcass weight/fatness ratio at slaughter (French et al., 2001). Many other studies reported no differences in quality and acceptability in meat from animals finished on pastures or concentrates (French et al., 2001; Mandell, Buchanan-Smith, & Campbell, 1998; Vestergaard et al., 2000). Other authors reported that forage-fed beef had equal or superior eating qualities including tenderness than concentrate-fed beef (Oltjen, Rumsey, & Putman, 1971; Realini, Duckett, Brito, Dalla Rizza, & de Mattos, 2004). This variability in results indicates that further research is necessary to establish the reasons for these differences.

The objective of this experiment was to evaluate the effect of different diets, including pastures with increasing levels of grain as supplement and a concentrate diet, on carcass and meat quality traits in Uruguayan steers.

2. Materials and methods

The study was run by the National Institute of Agricultural Research, at INIA La Estanzuela Research Station, Uruguay (Latitude 34°20'18" South, Longitude 57°41'24" West), over a period of 8 months (from September 2005 to April 2006).

2.1. Animals and diets

Eighty four Hereford steers with a common origin and background on pasture, (1.5 years old and 391 kg of live weight on average), were randomly assigned to one of the following diets:

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- (a) T1 – pasture: offered at 4% of live weight (LW).
- (b) T2 – pasture: offered at 3% of LW and grain (0.6% of LW).
- (c) T3 – pasture: offered at 3% of LW and grain (1.2% of LW).
- (d) T4 – concentrate and good quality alfalfa hay, offered *ad libitum*.

Corn grain was used in treatments 2 and 3. The concentrate diet was composed of 85% corn, 13% sunflower, 0.98% urea, and salt, minerals and vitamins. Alfalfa hay was chopped (2–3 cm) and provided with the concentrate. The pasture, composed of alfalfa (*Medicago sativa*), white clover (*Trifolium repens*) and fescue (*Festuca arundinacea*), was divided into nine plots by electric fencing, in which T1, T2 and T3 were released (three repetitions of seven animals per treatment). The intensive feed treatment (T4) was located in three open-air plots on concrete flooring. Animals from all treatments had *ad libitum* access to water and mineral salts.

Chemical composition of feeds offered, are reported in Table 1.

Pasture height, availability and quality were registered for each plot in 7-day grazing periods (pre and post grazing). The same data was collected for the pasture remaining in each plot. Botanical composition was registered throughout the experiment. Pasture results are not presented in this paper.

Animals were weighed early in the morning without previous fasting every 14 days. The grazing area for pasture-based treatments for the subsequent 7-days was calculated according to live weight and available dry matter. The grazing system was planned in order to avoid over-grazing and the corn grain was provided early in the morning in T2 and T3. Animals from T4 received the concentrate twice a day (morning and afternoon).

Ultrasound measurements were taken at intervals of 28 days, registering ribeye area and fat depth at the *Longissimus dorsi* muscle between the 12th and 13th rib, and fat depth in the rump area.

2.2. Slaughter and sampling procedures

Animals were slaughtered by humanitarian procedures in a commercial abattoir when they reached an average of 500 kg of LW in each treatment and at least 6 mm of fat covering (determined by ultrasound technique). Growth rates differed for all treatments and concentrate-fed animals (T4) were slaughtered on December 10th, 2005; followed by steers from T3 (December 27th, 2005), from T2 (January 24th, 2006) and from T1 on April 4th, 2006.

Carcasses were graded using the European Union (EU) beef carcass Classification System (EU Legislation, 1991) and the Uruguayan Grading System as specified by I.N.A.C. (1997). Both systems are based on conformation and fatness scores. Carcass conformation was based on a visual assessment of muscle mass development, with lower numbers indicating better conformation (1 = good muscle development and 6 = poor muscle development). The conformation score system for Uruguay is : I(1), N(2), A(3), C(4), U(5), R(6), and for the EU: S(1), E(2), U(3), R(4), O(5), P(6).

Fat finishing was based on the amount and distribution of subcutaneous fat, using a five grade scale and also subclasses, where lower numbers indicate lack of fat cover and higher numbers

excessive covering. The scores used by Uruguay are: 0, 1, 2, 3, 4, and by the EU: 1, 2, 3, 4, 5.

The Uruguayan system also includes a dentition scale from 1 to 4, based on the number of teeth. A lower score indicates a younger animal (1: *yearling steer* – without permanent incisors, 2: *young steer* – two to four permanent incisors, 3: *young steer with six teeth*, and 4: *steer* – eight permanent incisors).

Hot carcass weight (HCW) was registered. Carcass pH and temperature were measured at 1, 3 and 24 h post mortem (pm) at the *Longissimus dorsi* (LD) muscle between 12th and 13th rib, using a thermometer (Barnant 115) with Type E thermocouple and pH meter (Orion 210A) with gel device. At 24 h pm carcasses were ribbed between 5th and 6th rib, obtaining primal cuts. Subcutaneous fat thickness and fat colour were recorded at this time. The former was registered at the *L** (lightness), *a** (redness/greenness) and *b** (yellowness/blueness) colour space, using a colorimeter (Minolta C10) with an 8 mm diameter measurement area.

The pistola cut was prepared from the hindquarter by the removal of the thin flank, lateral portion ribs and a portion of the navel end brisket. A cut was made from the superficial inguinal lymph node, separating the *Rectus abdominus* and following the hip contour, running parallel to body of the vertebra and the *Longissimus dorsi* muscle (eye muscle) to the specified ribs.

The fabrication process was carried out according to a European commercial standard (United Kingdom). From the pistola cut, 7 boneless cuts were obtained (Striploin, Tenderloin, Rump, Topside, Silverside, Knuckle, Tail of rump), and the weights of trimmings and bones were recorded. Retail cuts were weighed and retail yield was calculated (pistola cut/hindquarter, 7 cuts/pistola cut; Rump&Loin,¹ R&L/pistola cut, others). Muscle, fat and bone percentages were calculated from the retail cuts, using the UK commercial standard (5% fat cover).

2.3. Meat quality

Two steaks (2.54 cm thickness) were vacuum packaged individually, transported to INIA Tacuarembó Meat Laboratory, and aged for 7 and 20 days at 2–4 °C. Meat colour, toughness and cooking loss were measured after each aging period.

Instrumental colour. Meat bags were opened and the exudation on the steak surface was removed with a paper towel. Muscle colour was measured on the LD after seven and twenty days of aging at the *L** (lightness), *a** (redness/greenness) and *b** (yellowness/blueness) colour space, using a colorimeter (Minolta C10) with an 8 mm diameter measurement area, after one hour of blooming. Values were registered from three different locations on the upper side of the steaks in order to obtain a representative average value of the meat colour.

Shear force. The LD steaks were placed inside polyethylene bags and cooked in water bath until an internal temperature of 70 °C was achieved, using a Barnant 115 thermometer with type E thermocouple. Six cores, 1.27 cm diameter, were removed from each steak parallel to the muscle fiber orientation. Shear force measurement (SF) was obtained for each core using Warner Bratzler (Model D 2000), and an average value was calculated for each steak.

Cooking loss. This was determined by the difference between the weight of the steak after cooking and its pre-cooked weight, shown as a percentage.

2.4. Statistical analysis

Exploratory analyses were performed with the Statgraphics and SAS programmes. Outliers were evaluated and eliminated. The

Table 1
Chemical composition of feeds offered

	OMD (%)	CP (%)	ADF (%)	NDF (%)	A (%)
Pasture	61.23	17.17	42.11	53.83	11.88
Corn grain	82.19	9.18	6.53	21.59	3.04
Concentrate	85.83	12.43	12.50	36.34	2.88
Alfalfa hay	57.03	23.26	33.40	39.54	11.11

OMD: organic matter digestibility, CP: crude protein, ADF: acid detergent fibre, NDF: neutral detergent fibre, A: ash.

¹ (striploin, tenderloin and rump).

relationship among variables was evaluated using Pearson correlations. Principal component analysis (PCA) was performed to describe the relationship between meat quality traits.

The statistical model applied was

$$y = t + b_1 I_w + b_2 F_w + e$$

where t is the treatment; I_w , the initial live weight; F_w , the final live weight and e is the error.

Final and initial live weights were used as covariates and the model included the treatment as a fixed effect. Interactions between final weight and treatment were eliminated, as they had no significant effect on the results obtained. Results were analysed by the GLM SAS procedure. LSM Means and differences between treatments were estimated.

With the exception of final live weight, conformation and finishing degree, all variables were adjusted for initial and final live weight.

3. Results and discussion

3.1. Animal performance and carcass traits

Concentrate-fed animals (T4) had the highest average daily gain (ADG), which increased with the level of energy in diet (Table 2). In general, when pastures and grains or concentrates are offered *ad libitum*, ADG is higher in concentrate-fed cattle, relative to grass-fed animals (French et al., 2000, 2001). Thus, concentrate-fed animals are younger when compared at a specific bodyweight or back-fat thickness. In our experiment, the use of high-energy diets, even when not *ad libitum* (T2 and T3), increased ADG and decreased days to slaughtering, involving different slaughter dates and consequently, animal age. Similar results were found by Gil

and Huertas (2001) when comparing animals from grain diets and high quality pastures. In our experiment, this produced a higher percentage of 4-teeth animals in the pasture treatment (T1). Forty percent of the former were 2-teeth while in the other

Concentrate-fed animals had higher final live weights (FLW) at slaughter (Table 2). No differences were found among other treatments in this variable.

Intermediate treatments (T2 and T3), had the higher hot carcass weight and T3 had the heaviest pistola cut. This information is consistent with that reported by Vaz Martins, Olivera, Cozzolino, Robaina, and Abraham (2003) when comparing pistola cut weight from pasture treatment and pasture plus grain (offered at 0.7% and 1.5% of LW), where treatment with grain offered at 1.5% of LW, showed the highest pistola cut weights. In this experiment, no differences were found among T1, T2 and T4 in pistola cut weight.

T3 also had higher weight of seven boneless cuts when compared with the pasture treatment (T1) and the concentrate (T4) (Table 2). Butterfield (1974) indicated that meat yield decreased as carcass weight increased (probably due to higher fat contents). However, in our experiment there were no differences in valuable cuts yield (meat yield), between T3 and the other treatments.

Striploin, tenderloin and rump (R&L) represent most of the commercial value of the carcass. Some markets require a certain weight for each cut. In this experiment, R&L weight and the retail yield of the R&L (rump&loin/hindquarter, rump&loin/pistola cut), did not show relevant differences between treatments.

Finishing strategies had an effect on carcass conformation and fat grading, according to the European and Uruguayan classification systems. According to the European Union Scheme (EU), carcasses from T1 had a poorer conformation than those from the other treatments, which did not differ among them. In pasture-fed animals, 45% of the carcass had an "O" conformation, while

Table 2

Effect of finishing strategy on daily gain, final live weight, hot carcass weight, pistola cut weight and boneless cuts weight General mean, residual typical deviation (σ_r), coefficient of variation (CV), difference between treatments, standard error (SE) and p -value

Characteristic	General mean	σ_r	CV	Contrast	Difference	SE	p -Value
Daily gain (kg)	1.18	0.045	3.79	T1-T2	-0.3237	0.014	<.0001
				T1-T3	0.5608	0.014	<.0001
				T1-T4	-0.8807	0.018	<.0001
				T2-T3	-0.2371	0.014	<.0001
				T2-T4	-0.557	0.018	<.0001
				T3-T4	-0.3199	0.018	<.0001
Final live weight (kg)	506.8	21.7	4.3	T1-T2	5.2928	6.967	0.4499
				T1-T3	-0.3927	6.949	0.9551
				T1-T4	-41.5424	7.078	<.0001
				T2-T3	-5.6855	6.885	0.4116
				T2-T4	-46.8352	6.953	<.0001
				T3-T4	-41.1497	7.001	<.0001
Hot carcass wt (kg)	267.99	6.835	2.55	T1-T2	-7.0014	2.204	0.0022
				T1-T3	-7.4566	2.19	0.0011
				T1-T4	-0.4433	2.706	0.8703
				T2-T3	-0.4552	2.18	0.8352
				T2-T4	6.5581	2.79	0.0215
				T3-T4	7.0133	2.678	0.0107
Pistola cut wt (kg)	64.34	2.108	3.28	T1-T2	-1.0948	0.68	0.1118
				T1-T3	-2.5919	0.676	0.0003
				T1-T4	-0.587	0.835	0.4843
				T2-T3	-1.497	0.673	0.0291
				T2-T4	0.5078	0.861	0.5571
				T3-T4	2.0049	0.826	0.0177
Seven boneless cuts wt (kg)	34.54	1.911	5.53	T1-T2	-0.6771	0.616	0.2757
				T1-T3	-1.5746	0.62	0.0133
				T1-T4	0.8359	0.76	0.2753
				T2-T3	-0.8974	0.618	0.1508
				T2-T4	1.513	0.784	0.0576
				T3-T4	2.4105	0.749	0.002

T1: Pastures, T2: Pastures + 0.6% grain of LW, T3: Pastures + 1.2% grain of LW, T4: concentrate *ad libitum* plus alfalfa hay.

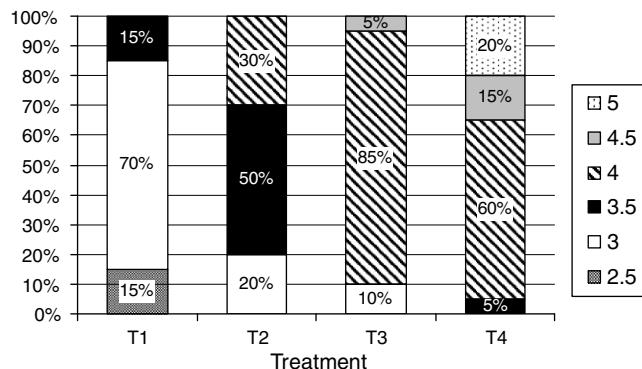


Fig. 1. Results of applying the EU Fat Grading system, by treatments: 0 = poorer finishing, 5 = excessive fat cover. T1: Pastures, T2: Pastures + 0.6% grain of LW, T3: Pastures + 1.2% grain of LW, T4: concentrate *ad libitum* plus alfalfa hay.

55% were “R”. Most of the other treatment carcasses were classified as “R”. High-energy diets during the finishing period, significantly improve carcass conformation (ÓFerrall & Keane, 1990).

Carcasses from T4 had higher fatness scores according to the EU system, 95% showing a score higher than 4 (Fig. 1). Only 30% of carcasses from T2 reached this score, and those from only pastures (T1) did not reach this figure. Eighty five percent of the former had a score of three or less. Results for only pasture-fed carcasses are similar to those reported by Montossi and Sañudo (2007) when evaluating Uruguayan steers with different ages (2 and 3-year-olds).

Ultrasonic final fat cover did not show relevant differences between treatments. Neither did subcutaneous fat (fat thickness) on the carcasses, show any difference between treatments, giving a general mean of 10.36, a residual typical deviation (σ_r) of 2.81 and 27.1 of coefficient of variation (CV). According to Robelin, Geay, and Beranguer (1979) the chronological order in fat deposition is intermuscular, internal, subcutaneous and intramuscular. This could explain differences obtained in fat percentage but not in fat cover. The finishing period in this experiment could have been enough to establish intermuscular and internal fat differences but not subcutaneous fat differences.

Muscle percentage did not show differences between treatments when pistola cut composition was evaluated, although many studies reported that a higher concentrate level during finishing periods leads to a lower proportion of muscle and bone in the carcass, along with a higher percentage of fat (Keane, ÓFerrall, & Connolly, 1989).

Pistolas from treatments with high-energy levels in diet (T3 and T4) had higher fat percentage than pistolas from T1 and T2 (Table 3). Similar results were obtained by other authors, reporting that fat proportion rose with concentrate-based diets (Micol, 1993). A fast growth rate caused by a high plane of nutrition, can lead to an earlier onset of the fattening phase of growth (Lawrie, 1998). In fact, in this experiment, ADG had a positive and significant correlation ($p < 0.001$, $r = 0.62$) with pistola fat percentage. Similar results were found by Cerdeño, Vieira, Serrano, Lavín, and Mantecón (2006). Fat ratio changed as long as ADG increased, due to the different planes of energy in the diet.

Pistola bone proportion did not show differences among T1, T2 and T3. However, they had higher bone percentages than the concentrate treatment (Table 3). Carcass composition is weight-dependent and largely influenced by age or nutritional regime. Other authors reported that even animals compared at a common weight will differ greatly in form and composition when they are on different levels of nutrition. Differences in carcass composition are mainly related to the differences in the energy density of the

Table 3

Fat and bone ratio in pistola cut General mean, residual typical deviation (σ_r), coefficient of variation (CV), difference between treatments, standard error (SE) and *p*-value

Characteristic	General mean	σ_r	CV	Contrast	Difference	SE	<i>p</i> -Value
Fat ^a (%)	0.104	0.017	16.7	T1-T2 T1-T3 T1-T4 T2-T3 T2-T4 T3-T4	0.0025 -0.0203 -0.0265 -0.0229 -0.0291 -0.0062	0.006 0.006 0.007 0.006 0.007 0.007	0.661 0.001 0 0 0 0.364
Bone (%)	0.2144	0.013	5.88	T1-T2 T1-T3 T1-T4 T2-T3 T2-T4 T3-T4	-0.0016 0.0038 0.0233 0.0054 0.025 0.0196	0.004 0.004 0.005 0.004 0.005 0.005	0.697 0.351 <0.000 0.185 <0.000 0

T1: Pastures, T2: Pastures + 0.6% grain of LW, T3: Pastures + 1.2% grain of LW, T4: concentrate *ad libitum* plus alfalfa hay.

^a Pistola fat + kidney fat.

diet and, in the end, to the total energy consumed (Cerdeño et al., 2006).

According to Sañudo (1997) higher carcass weight produces higher muscle thickness and fat deposits, which means the carcass and all its components have greater dimensions. In a relative value, higher carcass weight implies higher fat tissue and late mature zones, lower bone tissue and early mature components, and a more or less clear stabilization of muscle tissue and other isometric zones (those whose growth is proportional to the total growth).

3.2. Fat and meat quality

Grass-based treatments (T1, T2 and T3) had higher pH values than T4 (Table 4) at 1 and 3 h pm. Similar results were found by French et al. (2001), who reported that LD from pasture-fed animals had higher pH at 4, 5, 6, 7, and 8 h post slaughter when compared with LD from concentrate-fed cattle. This could be related to differences in muscle glycogen content, higher in animals fed with high-energy diets; and also to differences in stress susceptibility in

Table 4

Carcass pH at 1 3 and 24 h post mortem General mean, residual typical deviation (σ_r), coefficient of variation (CV), differences between treatments, standard error (SE) and *p*-value

Characteristic	General mean	σ_r	CV	Contrast	Difference	SE	<i>p</i> -Value
pH 1	6.48	0.121	1.86	T1-T2 T1-T3 T1-T4 T2-T3 T2-T4 T3-T4	-0.0693 0.057 0.3602 0.1263 0.4294 0.3031	0.0389 0.0386 0.0477 0.0384 0.0492 0.0472	0.0790 0.1441 <.0001 0.0016 <.0001 <.0001
pH 3	6.17	0.128	2.07	T1-T2 T1-T3 T1-T4 T2-T3 T2-T4 T3-T4	0.0037 0.1108 0.2688 0.1071 0.2651 0.158	0.0412 0.041 0.0506 0.0408 0.0522 0.0501	0.9293 0.0086 <.0001 0.0106 <.0001 0.0024
pH 24	5.61	0.117	2.08	T1-T2 T1-T3 T1-T4 T2-T3 T2-T4 T3-T4	0.1661 -0.0245 0.2348 -0.1906 0.0687 0.2592	0.0377 0.0374 0.0462 0.0372 0.0477 0.0458	<.0001 0.5151 <.0001 <.0001 0.154 <.0001

T1: Pastures, T2: Pastures + 0.6% grain of LW, T3: Pastures + 1.2% grain of LW, T4: concentrate *ad libitum* plus alfalfa hay.

pre-slaughter handling, higher in extensively reared animals. In the same way, pH values at 24 h pm were higher in carcasses from T1 than carcasses from T2 and T4 (Table 4). The pH decline depends on muscle glycogen concentration. Work by Daly, Richards, Gibson, Gardner, and Thompson (2002) showed that higher initial muscle glycogen concentration resulted in a faster rate of pH decline. The two major reasons for suboptimal glycogen levels at slaughter are a poor nutritional plane on farm (Pethick & Rowe, 1996) or excessive glycogen losses from stress during transport and lairage. According to Immonen, Schaefer, Puolanne, Kau, and Nordheim (2000) glycogen levels in muscle increase with metabolisable energy intake. These glycogen stores can then act as a buffer in the pre-slaughter period. These authors reported that the effect of a high-energy diet on the muscle content was reflected all the way to ultimate pH values and residual glycogen concentrations. Even a short finishing period of 2 weeks with a concentrate-based high-energy diet, was well worth applying because its clearly protective effects were directly against glycogen depletion and elevation of pH. Muir, Beaker, and Bown (1998) also reported that grass-fed steers had higher ultimate pH values than grain-fed ones, and they suggested that animals from pasture diets are more susceptible to pre-slaughter stress, so they could have had less glycogen content in muscle and consequently higher final and intermediates pH values than grain-fed steers. Feedlot steers are more used to penning and handling and would be less likely to suffer glycogen depletion during the pre-slaughter period. In this experiment, all animals were used to handling and penning because of frequent weighing, and a strict protocol of Good Management Practices was followed during the whole process. However, animals from T1 had less daily contact with humans. Other authors did not find differences in ultimate muscle pH in grain and grass-finished cattle (French et al., 2000, 2001; Morris, Purchas, & Burnham, 1997). These contradictory results could be partially explained by factors such as breed, animal temperament, management practices or just previous animal experience in handling.

In spite of the differences in initial and final pH values in our experiment, all were within the normal range and without incidence of dark cutting beef.

Carcasses from T1 had lower temperatures than the other three treatments at 24 h pm, T3 and T4 having the highest carcass temperatures (Table 5). This is consistent with results reported by Realini et al. (2004) when carcasses from pasture and concentrate diets were compared. The larger fat proportion obtained in pistola cuts from T3 and T4, could probably have an effect on post mortem chilling.

Carcasses from T1 had higher L^* fat values than carcasses from intermediate treatments and from T4 (Table 6). The same results were shown by Realini et al. (2004) when comparing grass and concentrate-fed animals.

Concentrate-fed animals had higher a^* fat values compared to the other three treatments, indicating more redness in subcutane-

Table 5

Carcass temperature at 24 h post mortem General mean, residual typical deviation (σ_r), coefficient of variation (CV), difference between treatments, Standard error (SE) and p-value

Characteristic	General mean	σ_r	CV	Contrast	Difference	SE	p-Value
Temp 24 (°C)	5.88	0.706	12	T1-T2	-2.3322	0.2277	<.0001
				T1-T3	-3.1924	0.2262	<.0001
				T1-T4	-3.4641	0.2796	<.0001
				T2-T3	-0.8602	0.2252	0.0003
				T2-T4	-1.1319	0.2882	0.0002
				T3-T4	-0.2717	0.2766	0.3293

T1: Pastures, T2: Pastures + 0.6% grain of LW, T3: Pastures + 1.2% grain of LW, T4: concentrate *ad libitum* plus alfalfa hay.

Table 6

Subcutaneous fat colour at 24 h post mortem General mean, residual typical deviation (σ_r), coefficient of variation (CV), difference between treatments, standard error (SE) and p-value

Characteristic	General mean	σ_r	CV	Contrast	Difference	SE	p-Value
<i>24 h post mortem</i>							
L^*	76.35	1.997	2.61	T1-T2	1.9263	0.6443	0.0038
				T1-T3	4.7703	0.6399	<.0001
				T1-T4	2.4746	0.7928	0.0026
				T2-T3	2.844	0.6373	<.0001
				T2-T4	0.5484	0.8163	0.5039
				T3-T4	-2.2956	0.7848	0.0046
a^*	5.01	1.341	26.77	T1-T2	-0.0682	0.4329	0.875
				T1-T3	0.1563	0.43	0.717
				T1-T4	-2.0634	0.5327	0
				T2-T3	0.2245	0.4282	0.602
				T2-T4	-1.9952	0.5484	0.001
				T3-T4	-2.2197	0.5273	<.0001
b^*	17.2	1.538	8.94	T1-T2	1.3143	0.4963	0.01
				T1-T3	1.862	0.493	0.0003
				T1-T4	3.229	0.6107	<.0001
				T2-T3	0.5477	0.4909	0.2684
				T2-T4	1.9147	0.6288	0.0033
				T3-T4	1.3671	0.6045	0.0268

T1: Pastures, T2: Pastures + 0.6% grain of LW, T3: Pastures + 1.2% grain of LW, T4: concentrate *ad libitum* plus alfalfa hay.

ous fat (Table 6). Kerth, Braden, Cox, Kerth, and Rankins (2007) did not find differences in L^* and a^* fat values when comparing grass and concentrate-fed steers.

b^* values were higher in T1 than in the other treatments. The most relevant difference was obtained when comparing T1–T4 (Table 6). Finishing strategy, pasture characteristics, animal growth rate and animal age at slaughter seem to have an effect on fat b^* value. Finishing cattle on pasture increased b^* values in subcutaneous fat, indicating more yellowness compared with fat from the other treatments. Other studies have consistently shown that feedlot-finished cattle have whiter fat colour than pasture-fed ones (Realini et al., 2004). Fat colour is strongly dependent on its carotenoid content. Green and fresh pastures usually contain high quantities of carotenoids (up to 550 ppm of dry matter), whereas most of the grains contain low carotenoid concentrations (<5 ppm) (Tume & Yang, 1996). Yang, Brewster, Lanari, and Tume (2002) demonstrated that β -carotene in plasma, muscle and adipose tissues increased the longer cattle graze on pastures. β -carotene levels increased by more than 50% in cattle finished on pastures compared with those finished on grain. Differences in the rate of fat deposition could also have contributed to differences in fat colour, due to different rates of dilution of the total carotenoid content (French et al., 2000). b^* values showed a reduction of 0.0364 units for each 1% of increase in the use of high-energy food in the diet (Fig. 2). However, Kerth et al. (2007) suggested that trimming subcutaneous fat from cuts from forage-fed cattle to 0.3 cm, reduce fat yellowness to similar levels to cuts from high-energy-fed cattle. Furthermore, carcass fabrication involves the trimming of almost all cuts nowadays. It seems that one of the most common complaints of pasture-finished beef in the United States and other countries seems not to be a problem any more, considering consumer acceptance (Kerth et al., 2007), although it could be a disadvantage in others that prefer white fat, such as Spain or Italy.

There was no effect of the diet on muscle colour after 7-days of aging. After 20 days, no differences were found in a^* meat values among treatments based on pastures (T1, T2 and T3), but all of them had higher a^* values than T4 (Table 7). Meat colour depends on myoglobin concentration and its chemical state, surface structure and intramuscular fat (Judge, Aberle, Forrest, Hedrich, & Mer-

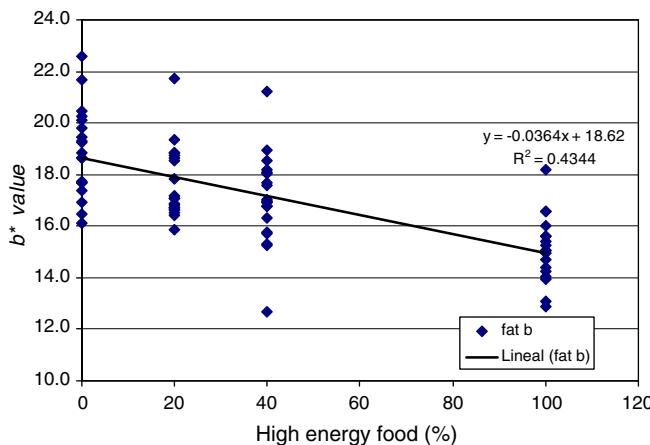


Fig. 2. Fat yellowness by increasing level of energy in the diet. T1: Pastures, T2: Pastures + 0.6% grain of LW, T3: Pastures + 1.2% grain of LW, T4: concentrate *ad libitum* plus alfalfa hay.

kel, 1989). Myoglobin is mainly responsible for meat colour, and a darker muscle (a^* value) could be attributed to higher myoglobin concentrations (Johansson, Agerhem, Magard M., & E., 1998). Diet, animal age and exercise could have an affect on meat colour. High-energy diets decrease hemin pigment concentrations and consequently reduce a^* values (Lawrie, 1998). Bennett et al. (1995) obtained similar results for a^* values when comparing forage-fed animals with concentrate-fed animals. As those fed forage were older at slaughter, these authors suggested that muscle colour increase with age. In this experiment, concentrate-fed animals reached slaughter end-point earlier than other treatments, so they could have had less myoglobin concentration due to their age. Moreover, all grass-based animals could have had higher muscle myoglobin concentration, due to more pre-slaughter activity than their concentrate counterparts (Varnam & Sutherland, 1995).

No differences were found in L^* values. Intermediate treatments produced higher b^* values than T4, but these differences were not considered relevant (economical criteria) (Table 7). Realini et al. (2004) indicated that pasture-fed animals had lower L^* beef values

Table 8

Shear force (WBSF, kg) after 7 and 20 aging days General mean, residual typical deviation (σ_r), coefficient of variation (CV), difference between treatments, standard error (SE) and *p*-value

Characteristic	General mean	σ_r	CV	Contrast	Difference	SE	<i>p</i> -Value
WBSF 7-days (N)	37.85	1.039	26.88	T1-T2	-0.9999	0.3437	0.0049
				T1-T3	-0.404	0.3375	0.2354
				T1-T4	-1.3377	0.4121	0.0018
				T2-T3	0.596	0.3344	0.0791
				T2-T4	-0.3378	0.4271	0.4316
WBSF 20 days (N)	33.93	0.668	19.28	T1-T2	-0.8552	0.2152	0.0002
				T1-T3	-0.2724	0.2167	0.2128
				T1-T4	-1.0914	0.2643	<.0001
				T2-T3	0.5828	0.216	0.0087
				T2-T4	-0.2361	0.2725	0.3892
				T3-T4	-0.8189	0.2646	0.0028

T1: Pastures, T2: Pastures + 0.6% grain of LW, T3: Pastures + 1.2% grain of LW, T4: concentrate *ad libitum* plus alfalfa hay.

than concentrate-fed ones, but did not find differences in a^* or b^* LD values. Several researchers did not find significant effects of different forage/concentrate ratio diets during finishing on muscle colour (Cerdeño et al., 2006; French et al., 2001). Some authors suggest that diet characteristics do not have capital relevance on meat colour, probably due to transformation processes that take place in the rumen (Albertí et al., 1992; Hedrick et al., 1983).

Shear force values were lower in T1 and T3 at 7-days of aging (Table 8). Differences were considered relevant only when comparing meat from the pasture treatment (T1) with that from the concentrate-based one (T4).

After 20 days of aging (Table 8), shear force followed the same pattern as with 7-days. Differences were relevant when comparing T1 with T4. This result is consistent with those obtained by Realini et al. (2004), who reported 2.8 and 3.5 kg (shear force values), for pasture and concentrate-fed animals, respectively. Also, Oltjen et al. (1971) reported that beef from steers fed on all-forage diet had higher tenderness values than beef from animals fed on all-concentrate diet.

Different external factors such as diet, pre-slaughter growth rate, animal age and the length of the finishing period, may affect beef tenderness. Finishing strategies in cattle have been extensively studied over the years with contradictory results on meat quality and carcass traits. Fishell, Aberle, Judge, and Perry (1985) reported that cattle with high growth rate prior to slaughter had more tender meat than those with slow growth rate. Higher ADG is supposed to increase rates of protein turnover, resulting in higher concentrations of proteolytic enzymes in the carcass tissue at slaughter (Miller, Cross, Crouse, & Jenkins, 1987) and more soluble collagen. Fast growing groups reach market weights earlier than slow growing animals, and are therefore younger at slaughter, within a market category. Age at slaughter has been shown to be related to meat tenderness (Perry & Thompson, 2005). However, several authors did not find any decrease in shear force, when comparing steers with different pre-slaughter growing rates (Cox et al., 2006; Moloney, Keane, Mooney, & Troy, 2000). Some authors indicated that meat quality differences between diets are minimised when forage-fed cattle are compared with concentrate-finished cattle at a common end-point such as body weight, fat thickness or degree of marbling (Crouse, Cross, & Seideman, 1984). Other authors reported that decreasing tenderness in forage-fed beef is related to the length of the finishing period (French et al., 2001). In this experiment, animals finished on pastures (216 days on treatment and with lower ADG) were more tender than the ones finished on feedlot (101 days on treatment and higher ADG).

Table 7

Meat colour L^* , a^* , b^* after 20 aging days General mean, residual typical deviation (σ_r), coefficient of variation (CV), difference between treatments, standard error (SE) and *p*-value

Characteristic	General mean	σ_r	CV	Contrast	Difference	SE	<i>p</i> -Value
L^*	39.77	1.619	4.069	T1-T2	-0.2007	0.5222	0.7019
				T1-T3	-1.0683	0.5255	0.0459
				T1-T4	-0.8466	0.6427	0.192
				T2-T3	-0.8676	0.5243	0.1024
				T2-T4	-0.6459	0.6616	0.3323
a^*	18.92	2.637	13.94	T3-T4	0.2217	0.6437	0.7316
				T1-T2	-0.0339	0.8505	0.9683
				T1-T3	-0.3218	0.8563	0.7082
				T1-T4	2.2868	1.0444	0.0318
				T2-T3	-0.2878	0.8538	0.737
b^*	11.02	1.243	11.28	T2-T4	2.3207	1.0767	0.0345
				T3-T4	2.6086	1.0456	0.0149
				T1-T2	-0.4607	0.4009	0.2543
				T1-T3	-0.6546	0.4036	0.1092
				T1-T4	0.728	0.4922	0.1436
				T2-T3	-0.194	0.4024	0.6313
				T2-T4	1.1886	0.5075	0.022
				T3-T4	1.3826	0.4928	0.0065

T1: Pastures, T2: Pastures + 0.6% grain of LW, T3: Pastures + 1.2% grain of LW, T4: concentrate *ad libitum* plus alfalfa hay.

There were no effects of dietary treatment on cooking losses at 7 and 20 days post slaughter. Similar results were found by other authors when comparing carcasses from different finishing strategies (Cerdeño et al., 2006; Kerth et al., 2007). However, Hedrick et al. (1983) reported higher cooking loss on grain-fed beef when compared with forage-fed cattle. These differences could be explained by the higher backfat registered by these authors with the higher energy diets.

3.3. Descriptive principal component analysis (PCA) for carcass, meat and fat quality traits

The first three PCs explained 71% of the total variation (31%, 24% and 16%, respectively), when performed on some carcass variables and meat quality data.

Fig. 3 shows a plot of traits for the two first PCs.

It was possible to define two groups of variables within the first PC and far from the origin, which means that they are predominant in defining this PC. One group included variables relative to the muscle-meat transformation process (pH decline) and also fat yel-

lowness. These variables were positively correlated to each other and negatively correlated to the other group composed of shear force at 7 and 20 days, and carcass temperature at 24 h pm. The second PC (Fig. 3) was an independent source of variation, mostly defined by meat colour on one side far from the origin, and on the other side by pH values, fat yellowness and shear force at 7 and 20 days of aging.

Four different types of points were seen when projecting all the data in the first two PCs (Fig. 4), but only two feeding strategies are clearly discriminated. Pasture fed animals were on the right side of the origin and grain-fed animals on the left. Intermediate treatments were not clearly differentiated. It is clear that T1 and T4 were mainly separated by shear force and fat yellowness.

4. Conclusions

Extensive production systems based mainly on pastures have been conventionally associated with some inferior meat quality attributes (tenderness, colour). However, in this study the finishing strategy did not show clear changes in meat quality. Thus, pastures finishing strategy not only did not clearly affect quality, but also increased beef tenderness. According to the experiment conditions, the use of concentrates to finish cattle is a matter of money and time, not a quality issue.

5. Implications

Feed costs are the most important factors in determining the commercial efficiency of any beef system. In certain regions, better grass utilization could be the cheapest source, especially considering fluctuations in grain prices. Commercial aspects that involve the use of concentrates and/or the extra time needed to finish animals with pasture should be taken into consideration with a view to shareholders' interests. Alternative methods for finishing cattle may be viable as long as they are profitable for producers, plant processors and they are accepted by consumers. To maximize profitability and attain different markets, potential animal growth could be achieved by an inclusion of concentrates in a forage-based diet, considering commercial issues and without any impairment of quality. Meat quality should include other aspects of increasing importance, such as fatty-acid composition (PUFA/SFA, n6/n3), oxidation rates of fat and pigments, cholesterol content, sensory quality (acceptability, intensity of odors and flavors, textural properties), animal welfare and sustainability. Some of them were analysed in this project and will be presented in others papers.

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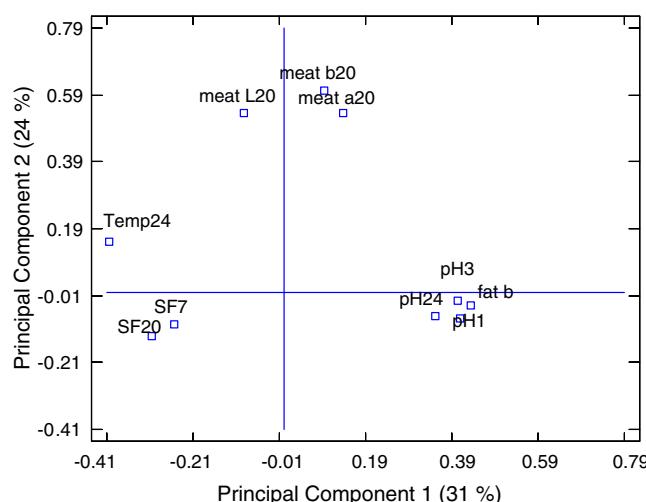


Fig. 3. Similarity map in a Principal Components assay, defined by PCA 1 (31%) and PCA 2 (24%). Abbreviations: Temp 24 – Temperature at 24 h post mortem; pH 1, 3, 24 – pH₁, pH₃ and 24 h post mortem; meat L₂₀, meat b₂₀, meat a₂₀ – lean colour L_a, a_b values, at 20 aging days; fat b – fat colour (b_{value}); SF 7, 14 – Shear force at 7 and 20 aging days.

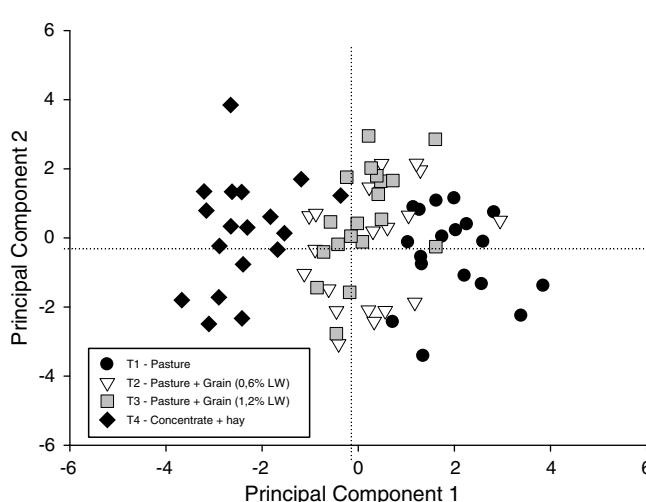


Fig. 4. Projection of all the data, in the space defined by the first two PCs.

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