Physical castration and inmunocastartion of early-naturing bulis ted high. Concetrate diets: welfare, performance, and carcass and meat quality

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Chapter I

LITERATURE REVIEW

1. INTRODUCTION

In Spain, one of the main beef production systems, is based on calves that arrive with 45-60 kg of BW at the farm and are fed during 9-12 month until 480-490 kg of BW concentrate and barley or wheat straw *ad libitum* (Bacha et al., 2005). The objective of this system is maximized the animal growth at minimum cost.

Catalunya sacrifices 22.1% of animals classified as 'ternera' of the total sacrificed in Spain (Instituto Nacional de Estadísica, 2011). This category includes heifers and bulls less than 12 mo of age. The 63 % of these animals are males and within these males 70 % are Holstein (Mach et al., 2008). In recent years, female production has decreased gradually (Micol et al., 2009) due to bluetongue disease and the increase of demand of female for milk production. Meat quality (marbling and tenderness) of females is better (Cahill, 1964) and for this reason meat price of heifers is greater in than that of meat of Holstein males (3.50 vs. 3.70 €/kg, Mercabarna, 2012). So, there is an interest to produce male animals with a similar meat quality as females, and this could increase the meat prices and help to increase the Holstein male producer's benefits. Castration could be a good alternative to improve meat quality of Holstein bulls as described by Mach et al. (2009). In this study (Mach et al., 2009) Burdizzo castration applied to 8 mo old Holstein bulls improved meat quality and reduced aggressive and sexual behavior, however the castration required labor, was difficult to perform under commercial conditions, and rates of failure were great. For this reason, and encouraged by the meat quality improvement and better animal handling obtained by the castration of Holstein bulls in Mach et al. (2009) study, alternative castration methods have been evaluated. During the current

Thesis these alternative methods have been evaluated from different point of views; their effect on animal welfare, on performance and on meat quality.

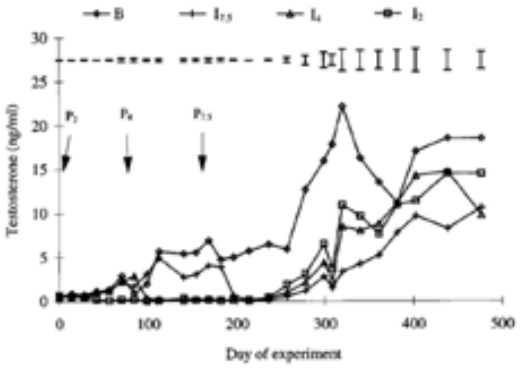
In the following paragraphs a short review summarizing the castration methods and castration ages and their consequences on animal welfare, performance and meat quality are presented.

2. CASTRATION

Castration is defined as the removal of testes (Encyclopedia Britannica, 2012). In animal husbandry traditionally castration was performed by the removal of the testes or the testes blood-flow suppression. Actually, new methods are available like the vaccine anti-GnRH or anti-LHRH, these methods are not physical methods that cause a mutilation as the traditional one, but also cause a suppression of serum testosterone levels (this suppression is temporary). So, castration needs to be redefined. Nowadays, castration could be defined as the application of a method that reduces during a prolonged time serum testosterone concentration below 5 ng/mL. This thereshold serum testosterone concentration (< 5 ng/mL) has been defined based on the effect of immunocastration on serum testosterone concentration and its impact on sexual and aggressive behavior (Jago et al., 1997; Huxsoll et al., 1998; Price et al., 2003; Figure 1), testes size, and meat quality (Cook et al. 2000; Amayatakul-Chantler et al., 2012).

There is also a great confusion with the terminology that describes a castrated animal. A bull is an intact adult male, in some papers it is specified that bull is an intact animal to compare it with a castrated animal. A castrated bull in the USA and Canada is usually described as a steer; however, when castration is performed during the study these animals can be refered as “castrated animal” instead of steer.

Figure 1. Relationship between serum testosterone concentration and mean sexual behavior score (0 = no interest, 6 =serve cow) in bulls (B), bulls immunized against GnRH at 2, 2.5,4, and 7.5 mo of age (I2), at 4, 4.5 and 7.5 mo of age (I4), and 7.5 and 8 mo of age (I7.5), and steers (S) castrated at 2 mo of age (Jago et al., 1997).



	Treatment				
Test (d)	B	I_{comb}[a]	S	SED[b]	SED[c]
1 (161)	1.5[x]	1.2[x,y]	.9[y]	.277	.240
2 (203)	2.0[x]	1.7[x]	.5[y]	.654	.534
3 (246)	2.7[x]	1.2[y]	.8[y]	.677	.552
4 (291)	4.1[x]	2.2[y]	1.6[z]	.573	.468
5 (342)	4.7[x]	2.3[y]	1.2[y]	.692	.565
6 (450–455)	5.4[x]	3.7[y]	1.8[z]	.836	.623

[a]There were no differences between treatments I_2, I_4, and $I_{7.5}$, so data from these treatments were combined and are presented as I_{comb}.
[b]SED (standard error of the difference) calculated for (minimum) comparisons between B (n = 10) and S (n = 10).
[c]SED calculated for (maximum – minimum) comparisons between I_{comb} (n = 30) and B (n = 10) or S (n = 10).
[x,y,z]Means within a row without common superscripts differ ($P < .05$).

2.1. Castration Methods

2.1.1. Physical castration

In general two methods of castration are considered, open castration and close castration. In an open castration testicles are removed after an incision in the scrotum, also this method is called surgical castration. Stafford et al. (2002), differenced two techniques of surgical castration, the pull one where the spermatic cord is broken due to traction, and the cut one, in this case the spermatic cord is cut down with an emasculator. Surgical castration is associated with infections and bleeding (Turner and McIlwraith, 1989) and in some cases with the death of the animal (Gregory and Ford, 1983; Vanderwert et al., 1985).

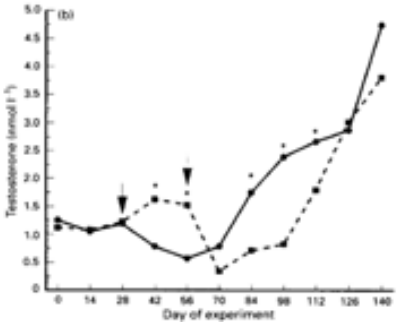
The close castration can be performed using an emasculator, where the spermatic cord is cramped and blood-flow is suppressed (Burdizzo). Also close castration can be performed using a rubber-ring or bands, in this case an ischemia followed by necrosis is produced until the testes fall-down. Burdizzo technique needs an accurate management because the correct utilization of the emasculator is the key to avoid incomplete

castrations (Boesch et al., 2008). Although is a quick and an economic technique (Zweiacher et al., 1979), this method has the risk to be incomplete; Mach et al. (2009) observed round 23% of incomplete castrations with the utilization of Burdizzo method. The rubber-ring and bands have a similar effect to Burdizzo castration, however it includes the hypoxia and anoxia of the scrotum causing the death of the tissue, which later causes the detachment of the testes from the abdominal wall. Some authors indicate that the pressure exerted by bands is greater than the pressure produced by rubber-rings (Stafford et al., 2002); however the use of rubber-rings is limited to animals of 3-4 month of age.

2.2.1. Immunocastration

Immunocastation against-LHRH or against-GnRH has been long recognized as a key hormonal target for preventing reproduction in livestock decreasing LH, FSH, which are necessary for androgen production and spermatogenesis (Jago et al., 1997; Huxsoll et al., 1998). This reduction in serum testosterone concentration causes a similar effect to physical castration (Robertson et al., 1979). However, the effect of immunocastration is temporary and reversible (Figure 2). The success of immunocastration (period of serum testosterone concentration suppression and its benefits on behavior and meat quality) depends on the age at the first vaccination, number of vaccinations, the interval between vaccinations, the adjuvant type, and the breed and production system (Finnerty et al., 1994).

Figure 2. The effect of a primary-booster interval of 28 days (●) or 56 days (■) on testosterone concentrations of bull calves (n = 24 per group) actively immunized against 0.1 or 1.0 mg of a GnRH analogue—human serum albumin (HSA-Cys-GIy-GnRH) conjugate. Arrows indicate times of booster injection. *Within day, mean hormone concentrations are significantly different (P < 0.05; Finnerty et al., 1994)



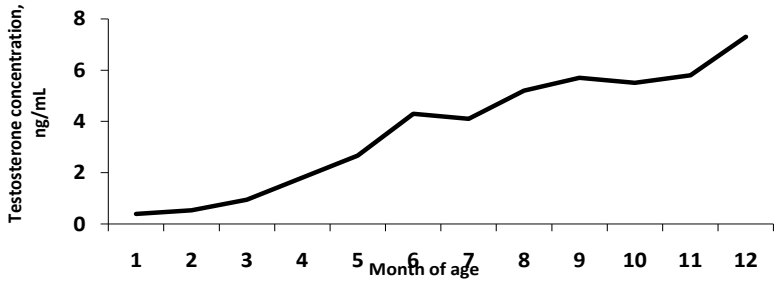
2.2. Castration age

The major differences in performance between intact bulls and steers are observed post-puberty (Keane, 1999). During the puberty androgens are produced by testes, mostly testosterone (Arey, 1965; Henricks, 1991; Figure 3). Androgens are responsible of the development of male organs, secondary sexual characteristics and male behavior (Sadleir, 1973). Moreover, androgens promote the muscular development by the increase of nitrogen retention (Galbraith et al., 1978; van Tienhoven, 1983). This anabolic property is related with the growth (Bretschneider, 2005), intact bulls grow 14 to 19 % more than steers being more efficient (Brännäng et al., 1966; Hedrick, 1969; Field, 1971; Knight et al., 1999).

Castration age is an important parameter to consider from the welfare point of view and from the production point of view. Mellor et al. (1991) observed that stress response to castration decreased when bulls were castrated at ages lower than 6 month of age due to the less testicular development of these younger animals. In addition, Bretschneider (2005) indicated after a literature revision that castration after birth had minimal impact on the weight loses associated to castration pain. Robertson et al. (1994) and Boesch et al.

(2008) also observed less abnormal postures and activities in animals castrated at early ages.

Figure 3. Evolution of serum testosterone concentration in beef bulls between 1 and 12 mo (Lunstra et al., 1978; Aman and Walker, 1987)



Boesch et al. (2008) proposed two theories to justify the less pain response to castration in young animals compared to older animals. In the first one, these authors suggested that very young calves might not perceive pain because of an incompletely developed nervous system, which could be interpreted as an adaption to the marked, albeit normal, physical stress of parturition. And a second explanation could be that very young calves instinctively fail to exhibit a behavioral response to a painful stimulus led to the cortex. Again, this could be an adaptive mechanism, because during the first days of life, calves typically rest away from the herd and a behavior indicative of pain may alert predators.

Castration age also is important to decide which methods should be used. Post-pubertal castration is limited to surgical castration and band castration (Chase et al., 1995). However, Bretschneider (2005) indicated that surgical castration after puberty has an important detrimental effect on performance, which would not permit the steers to keep the advantage on weight gain achieved by the growth-enhancing properties of testosterone. In addition, Fisher et al. (2001) demonstrated that cattle castrated by rubber

bands had a slower growth than those surgically castrated due to a prolonged wound resolution.

Knight et al. (1999) proposed post-pubertal castration to approach the production benefits of intact bulls until castration and after post-pubertal castration the benefits on meat quality of castrated animals. In other production systems where animals graze with herds until weaning (6-9 month old), when castration is performed at weaning the weight loss increases, implying that they have a weight disadvantage with respect to those calves castrated at birth (Champagne et al., 1969; Worrell et al., 1987). Moreover, Devant et al. (2012) observed a great detrimental effect on performance in animals surgically castrated at post-pubertal ages. Therefore, it is not clear that post-pubertal castration is a good strategy to improve weight gain compared to pre-pubertal castration (Jago et al., 1996; Fisher et al., 2001).

2.3. Welfare and castration

Welfare is a concept with different definitions. It can be defined throughout of animal emotions, according to the environment adaptation and the capacity to have a normal behavior. These changes can be (objectively) measured and can be analyzed as welfare indicators like decrease of growth, body damage and illness, problems in reproduction function, increase in abnormal behavior and reduction in immune response. One of the most important causes that alter these welfare indicators is pain; Broom (1991) defined pain as a sensation that is extremely averesive. Measurement of pain is difficult, however, Morton and Griffiths (1985) pointed out that careful measurement of behavior can be a good indication of the extent of pain.

Pain can be classified as an acute pain when is produced in a short term and the origin of it is easy to indentify, or chronic pain, when the cause of it normally is independent of the original pain, and the exalted nervous continously sending pain signals to brain.

Castration is considered one of the most painful experiences for calves and is questioned form the welfare point of view because these animals suffer pain, physiologic stress, inflammatory reactions, immune response suppression and performance is reduced (Fisher et al., 1997; Pang et al., 2006). However, depending on the method of castration and age when castration is performed, the pain suffered can be different (Stafford et al., 2002), as discussed previously.

Welfare evaluation is complex and it does not exist a standardized protocol. There is no method to evaluate pain directly, and physiologic or behavior parameters are used no standardized parameters are used in the different studies where castration effects on welfare are evaluated (Table 1). Robertson et al. (1994) and Molony et al. (1995) used changes in behavior and serum cortisol concentrations to evaluate the effect of different castration methods on pain, whereas Stafford et al. (2002) only used serum cortisol concentration, Fisher et al. (1997) and Early and Crowe (2002) used serum fribrinogen and haptoglobin to evaluate the effects of castration on chronic pain. In additon, Thuër et al. (2007) and Molony et al. (1995) also scored the lesions produced by castration or González et al. (2010) analyzed feeding behavior after castration as a welfare indicator.

2.3.1. Welfare indicators

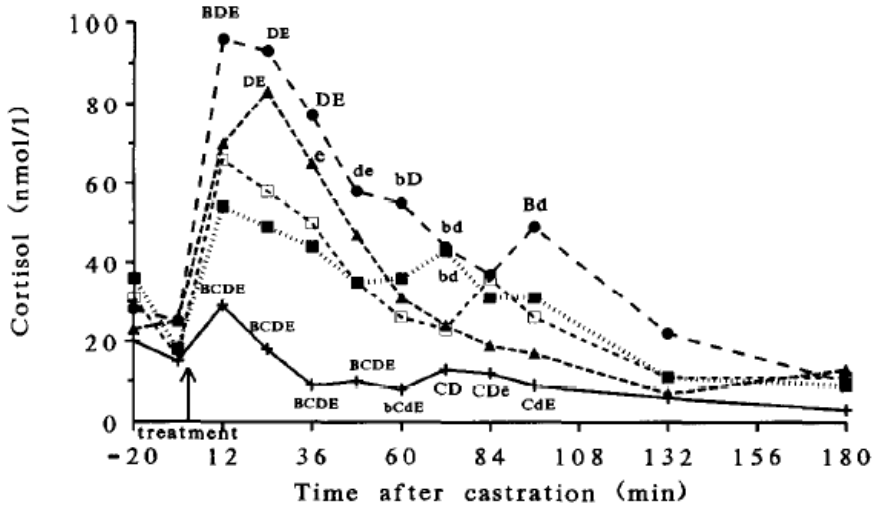
One of the most common parameter used to evaluate welfare is performance (growth and intake), one of the first signs of discomfort is the decrease of feed intake. Growth

reduction can be produced by the tissue damage due to castration and by the physiologic stress (Pang et al. 2008), but also could be can be produced by the supression of anabolic hormones (Knight et al. 1999) or the decrease in feed consumption. Usually, when castration causes an acute pain, the decrease observed in intake and performance takes place during 1-3 weeks after castration; after this period if growth is reduced but intake is not reduced, is not clear if the growth reduction is due to pain/stress or due to the abscence of anabolic hormones. Fisher et al. (1996) and Fisher et al. (1997) observed that Burdizzo and surgical castration reduced intake during 5 and 7 d post-castration, respectively, compared with bulls. Chase et al. (1995) and Fisher et al. (1997) observed in band- and surgically castrated animals an impairement in ADG during the first 21 d post-castration. Pang et al. (2006; 2008) observed that the reduction in ADG was greater in band-castrated animals than Burdizzo-castrated animals, and it is negative effect on ADG was longer band-castrated animals than in Burdizzo-castrated animals. However, Fisher et al. (1996) did not observed differences in ADG between immunocastrated animals and bulls.

Castration is a practice that causes physiologic changes (Pange et al. 2006; Rushen et al. 2008) indicating that animals suffer pain, fear or ansiety. Since decades, the increase in the secretion of cortisol has been related to stress or pain (Moberg and Mench, 2000). The measures of cortisol seems to give fiable information about the activity of hypotalamic-pituitary-adrenal axis after an acute pain (Rushen et al. 2008), however it is questioned as an indicator of chronic pain. Some authors indicated that serum cortisol concentration raises immediately after castration and that it was greater in animals castrated with Burdizzo (Fisher et al., 1996; Stafford et al., 2002; Thüer et al., 2007) or surgically (Fisher et al., 1996; Stafford et al., 2002) than in animals castrated with bands or rubber-rings (Figure 4). However, after some hours post-castration, in band- and

rubber-ring- castrated animals, serum cortisol concentration was greater than Burdizzo- or surgically castrated animals (Chase et al., 1995; Pang et al., 2006; Thüer et al., 2007).

Figure 4. Effect of handling (H) and Burdizzo (Bu), surgical (S), combined Burdizzo rubber-ring (Brr) and rubber-ring (RR) methods of castration on plasma cortisol values of 5- to 7-day-old calves. ↑Treatment: + - + , H; ▲--▲, Bu; ●- - -●, S; □-□, Brr; ■||| ■, RR. For comparison of methods, in one direction only: upper case superscripts (P <0.0l); lower case superscripts (P < 0.05). B/b indicates difference to Bu group. C/c indicates difference to S group. D/d indicates difference to Brr group. E/e indicates difference to RR group (Molony et al. 1995).



In recent years, a new method to evaluate chonic pain has been proposed. The detection of cortisol in hair was evaluated by Koren et al. (2002) in wild animals. More recently Comin et al. (2011) evaluated the effect of switching the animals from winter housing to summer highland grazing on hair cortisol levels. There are various advantages of using cortisol in hair, this method permits to do a retrospective examination of cortisol at the times when a stressor was most slient, without needing to take a sample right at the time, and the sample can be collected noninvasively by simply cutting; this eliminates the risk that the sampling itself may have an impact upon cortisol production (Russell et al. 2012). However, no published studies are available where the effect of castration on hair cortisol has been evaluated.

The acute phase proteins are a group of blood proteins that change in concentration when animals are subjected to external or internal challenges, such as infection, inflamation, surgical trauma or stress (Murata et al., 2004). There are different kind of acute phase proteins: fibrinogen is commontly used in ruminants as indicatior of inflammation, bacteriological infection or post-surgical trauma (Hirvonen and Pyorala, 1998); in ruminants serum haptoglobin circulating levels are negligible, therefore it's a good indicator of chonic inflammation and tissue damage (Horadagoda et al., 1999); and ceruloplasmin is a good indicator of infection in cattle (Conner et al., 1986). When acute phase proteins have been used as indicators of tissue damage, usually a combination of several acute phase proteins are analyzed (Fisher et al., 1997; Earley and Crowe, 2002; Ting et al., 2003a i 2003b; Pang et al., 2006; 2008); for example, Pang et al. (2006) did not observe differences in serum fibrinogen concentration between Burdizzo- and band-castrated animals, however serum haptoglobin concentration was greater form d 3 to d 35 post-castration in band-castrated animals compared with Burdizzo-castrated animals.

As commented previously, behavior may be a good welfare indicator. Castration causes changes in behavior due to the pain produced by the mutilation (Rushen et al., 2008). Molony et al. (1995) evaluated the effect of different castration methods on behavior (Burdizzo, surgical, rubber-rings and rubber-rings combined with Burdizzo) and observed that rubber-rings increased the active behavior and abnormal lying postures 3 h post-castration compared with the other castration methods; Similar results were observed by Thüer et al. (2007). Moreover, all castration methods evaluated by Moloney et al. (1995) increased abnormal standing postures after castration. However, when the effect of castration on behavior was evaluated after a long period (48 d after castration), rubber-ring-castrated animals had a greater incidence of scrotal zone licking and abnormal standing postures, and Burdizzo-castrated animals had the greatest percentage of total

abnormal postures compared with the other castration methods, in contrast to the results obtained by Thüer et al. (2007).

There are other indicators to evaluate pain as a pain scale proposed by Molony et al. (1995) or Thüer et al (2007) that includes the observation and palpation of the mutilated zone. However, different authors observed some contradictory results, whereas Robertson et al. (1994) described Burdizzo as a method that caused less response to pain, Thüer et al. (2007) described Burdizzo as the method that had the major impact on pain scoring during palpation.

Rectal temperature can also be an indicator of welfare because tissue inflammation or infections can produce fever. Pang et al. (2008) observed that rectal temperature in band-castrated animals was greater than in Burdizzo-castrated animals. However, Ting et al. (2003a) did not observe an increase on rectal temperature after surgical castration. Moreover, the scrotal temperature is useful to evaluate if band and ring castration is well performed, because these methods supress the blood circulation reducing the scrotal temperature. In contrast, usually scrotal temperature after Burdizzo castration increases during the inflammation process (Pang et al. 2008).

Nowadays is also common to evaluate the respone of the immune system to evaluate stress (Moberg and Mench, 2000), usually in the evaluation of castration, celular immune response is evaluated. Castration did not affect serum interferon γ concentration that was used to determine if castration compromised the immune capacity of calves (Fisher et al., 1997; Earley and Crowe, 2002; Ting et al. 2003a and 2003b; Pang et al., 2006). No published studies are available where the effect of castration on humoral immune response has been determined (for example, antibodies against ovalbumin).

Table 1. Review of castration age, method and welfare indicators.

Authors	Age	Method[a]	Performance	Cortisol[c]	Acute phase proteins[b]	Behavior	Immune response	Lesion score	Hematology	Testes evaluation
Zweiacher et al., 1979	-	BAND/BURD	X							
Faulkner et al., 1992	6-9 mo	S	X							
Robertson et al., 1994	6-21-42 d	BURD/RR/S				X				
Chase et al., 1995	20-21 mo	BAND/S	X	X_4					X	
Molony et al., 1995	1 wk	BURD/RR/S	X			X		X		
Fisher et al., 1996	5,5 mo	BURD/S	X	X_4						X
Fisher et al., 1997	5,5 mo	S	X	X_4	$X_{1,2}$		X	X		
Earley and Crowe, 2002	5,5 mo	S	X	X_4	$X_{1,2}$		X		X	
Stafford et al., 2002	2-4 mo	BURD/BAND/RR/S		X_4						
Ting et al., 2003a	11 mo	S	X	X_4	$X_{1,2}$	X	X		X	X
Ting et al., 2003b	9 mo	BURD	X	X_4	X_1		X		X	
Pang et al., 2006	5,5 mo	BURD/BAND		X_4	X_1		X			
Thüer et al., 2007	21-28 d	BURD/RR		X_4		X		X		
Boesch et al., 2008	2-7 d	BURD		X_4		X		X		
Pang et al., 2008	12 mo	BURD/BAND	X		X				X	X
González et al. 2010	7m o	BAND	X	X_5						
Warnock et al., 2012	7 mo	SUR/BAND	X		$X_{1,3}$					

[a] Castration method: BAND= bands, BURD= Burdizzo, RR= rubber-ring, S= surgical.
[b] Serum actue phase proteins: 1: Haptoglobin, 2: Fibrinogen 3:Ceruloplasmin.
[c] 4: serum; 5: saliva.

2.3.2. Pain mitigation in castration

When anesthesia is applied, it is expected that the animal feels insensitivity. Local anesthesia locally inhibits action potentials in nerve cells by inhibiting sodium influx through the nerve cells member (McCormarck, 1994). Although anesthesia does not achieve a completely painless castration, anesthesia before castration has a certain effect reducing the response to local palpation (Boesch et al., 2008); minimizing the cortisol concentration levels after castration (Fisher et al., 1996; Early and Crowe, 2002; Stafford et al., 2002); reducing abnormal postures (Robertson et al., 1994; Molony et al., 1995; Thuër et al., 2007). In addition, the use of anesthesia also reduces the ADG losses after castration (Fisher et al., 1996). The provision of local anesthesia before castration is a legislative requirement in some countries (Albraight, 1983).

Systemic administration of NSAID has been shown to act both centrally and peripherally, with central actions to be releated to supraspinal effects cusing inhibiton of spinal transmition of nociceptive inputs (McCormarck, 1994). Different authors have also evaluated the effect of analgesia use on welfare indicators after a castration. Analgesia at castration reduced the serum cortisol response and serum concentrations of acute phase proteins (Early and Crowe, 2002; Ting et al., 2003a). Pang et al. (2006) evaluated the effect of carprofen alone in band- and Burdizzo-castrated animals, these authors observed that systemic analgesia using carprofen failed to suppress the initial serum cortisol rise (0 to 6 h post-castration), but reduced acute phase protein concentration following castration.

The effect of the combination of anaesthesia and analgesia on the reduction of pain after castration is not clear. Early and Crowe (2002) observed that the initial peak of serum cortisol concentration after surgical castration was suppressed by the

administration of anesthesia but ones of the effect of local anesthesia wore off (75 min), the use of ketoprofen continued to suppress cortisol concentration suggesting that analgesia was effective modulating the cortisol response. Suporting these authors observations, Ting et al. (2003a) proposed the use of analgesics should be considered as an alternative therapeutic or adjunt to local anesthesia to achieve a more balanced analgesia during castration. In contrast, Stafford et al. (2002) injected lignocaine into each testis and the distal scrotum 20 min prior to ring castration suppressing the acute serum cortisol raise; no additional effects of the ketoprofen administration were observed. Therefore, local anesthesia reduces the acute pain produced by castration and it is not clear if the combination of anesthesia with analgesia may help to reduce the acute pain and chonic pain after castration. Futher research is necessary to evaluate different anesthesia and analegesia protocols (products, route of administration, doses) for the different castration techniques performed at different ages.

2.3.3. Welfare Legislation on Castration

In the EU there is a specific legislation for pig castration (Directive 2001/88/EC), however for ruminants there are only Recommendations concerning the protection of ruminants kept for farm proposes adopted by the Permanent Committee in the 17th meeting (October 21st, 1988). In these Recommendations it is indicated in Article 17th that castration of bulls and bull-calves should be avoided where possible, but maybe carried out under local or general anesthesia by a veterinary surgeon or any other person qualified with domestic legislation. The Council of Europe recommends surgical castration as a method to castrate because the other methods cause unnecessary or prolonged pain and distress.

Table 2. Review of age, castration method, use of anesthesia and analgeisa.

Author	Age	Method[a]	Anesthesia	Dosis	Time	Place	Analgesia	Dosis	Time	Place[b]
Zweiacher et al., 1979	-	BAND/BURD	-				-			
Faulkner et al., 1992	6-9 m	S	Xylazine	0,02 mg/kg	-90 s	IM	Butophanol	0,07 mg/kg	-90 s	IM
Robertson et al., 1994	6-21-42 d	BURD/RR/S								
Chase et al., 1995	20-21 m	S	Lidocaine	25 ml	-3 min	S.E.	-			
Molony et al., 1995	1 w	BURD/RR/S	-				-			
Fisher et al., 1996	5,5 m	BURD/S	Lidocaine	8 + 3 mL	-15 min	S.E. + S	-			
Fisher et al., 1997	5,5 m	S	-				-			
Earley and Crowe, 2002	5,5 m	S	Lidocaine	6 + 3 mL	-20 min	S.E. + S	Ketoprofen	3 mg/kg	-20 min	IV
Stafford et al., 2002	2-4 m	BURD/BAND/RR/S	Lidocaine	3 + 2 mL	-30 min	T + S	Ketoprofen	3 mg/kg	-30 min	IM
Ting et al., 2003a	11 m	S	-				Ketorprofen	1,5 /3 mg/kg	-20 / 0 min/ 24 h	IM
Ting et al., 2003b	9 m	BURD	-				-			
Pang et al., 2006	5,5 m	BURD/BAND	-				Carpofren	1,4 mg/Kg	-20 min	IM
Thüer et al., 2007	21-28 d	BURD/RR	Lidocaine	10 mL	-5 min	S.E. + S	-			
Boesch et al., 2008	2-7 d	BURD	Lidocaine / Bupivacaine	2 + 1,5 mL		S.E. + S				
Pang et al., 2008	12 m	BURD/BAND	Lidocaine	6 + 6 mL	-15 min	T+ S				

[a] Castration method: BAND= bands, BURD= Burdizzo, RR= rubber-ring, S= surgical.
[b] Place: IM= Intramuscular, S.E.= spermatic cord, S= srotal, T= Testicle, IV= Via intravenosa

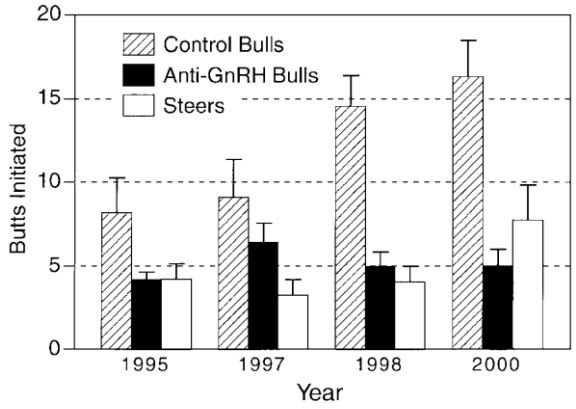
The European Community legislation concerning the welfare conditions of farm animals lays down minimum standards. National governments may adopt more stringent rules. In some countries, like Switzerland, Austria or United Kingdom, the use of anesthesia is required to perform castrations (Thüer et al., 2007). Spain does not have a specific legislation on castration. Moreover, organic production (CEE 889/2008) legislation permits the use of physical castration to improve meat quality and traditional practices, only when pain mitigation procedures are applied and a veterinarian performs it.

2.4. Sexual and aggressive behavior and castration

Bull calves become sexually active before they reach sexual maturity and their pursuing and mounting may cause management problems and carcass bruising. At 4 and 6 mo of age, intact bulls mount regularly (Wolf et al. 1965; Bass et al. 1977). Generally, castration decreases the frequency of sexual and aggressive behavior (Katz, 2007), and it improves the handling in farms. However, Imwalle and Schillo (2002) observed that castration did not suppress the mounting behavior at all, suggesting that the expression of this behavior may not be only dependent on serum testosterone concentration. But these authors suggested that may be 4 wks after castration was not enough time to observe a reduction of mounting. Supporting this hypothesis, Mach et al. (2009), observed that mounting behavior was reduced from 60 to 121 d after castration.

Mounting is one of the most common sexual behavior evaluated, but other sexual behavior as flehmen (Imwalle and Schillo, 2002) or aggressive behavior as fighting or butts (Price et al., 2003; Figure 5) are also reduced by castration and well correlated with serum testosterone levels (Imwalle and Schillo, 2002).

Figure 5. Mean (±SE) number of sparring bouts individual nonimmunized bulls, bulls immunized against GnRH and steers participated in during five, 20-min exposures to a novel arena in each of four years (Price et al. 2003).



Immunocastration reduced on aggressive and sexual behavior (Jago et al., 1997; Huxsoll et al., 1998; Price et al., 2003) when serum testosterone concentrations were below 5 ng/mL, for this reason these authors propose immunocastration as good alternative to surgical castration.

2.5. Long-term performance and Castration

It is known that castration produces a decrease on ADG and weight losses beyond the possible ADG and feed intake reduction during the first weeks after castration related mainly to the pain or stress. Numerous research reports have clearly shown the advantage of bulls in ADG compared to steers. The less growth rate of castrated animals compared to intact bulls seems to be due to a reduction of natural anabolic homones production by the testes (Adams et al., 1996; Knight et al., 1999; Mach et al., 2009) that promotes the muscular development and nitrogen retention (Galbraith et al., 1978; van Tienhoven; 1983) and due to an increase of fat accretition (Berg and Butterfield, 1966; Champagne et al., 1969; Hedrick et al., 1969). In fact, Fisher et al. (2001) administrated exogenous testosterone to castrated animals to investigate the roles of testosterone and castration in animal growth, but the exogenous testosterone administered was insufficient to increase

plasma testosterone to the levels of intact calves and its effect on growth was minimal. Replacement of testosterone (using exogenous treatment) in castrated animals to levels equivalent to those of intact calves would help in the elucidation of the effects of the lack of testosterone in castrated animals on growth.

In the literature reviews (Brännäng et al., 1966; Field, 1971) it's summarized that intact bulls grow 14 to 17 % more than steers being more efficient. In the literature summarized in Table 3 similar results are found; bulls grow 20% (from 6.2 to 26.2 %) more than castrated animals, however is not easy to compare the effect of castration on performance among studies. The estimated percentage in ADG loses when animals are castrated varies depending on factors like the possible BW reduction after the castration due to pain/stress, application of pain mitigation protocols, feeding programs, castration method, implants, days of study, interval between castration and slaughter, etc. In some studies (Champagne et al. 1969; Table 3), animals castrated at different ages start the study with the same initial BW, in others (Knight et al., 1999) animals castrated at different ages start the study with different BW, and the estimation of the effect of castration on overall ADG is difficult to calculate. So, the interpretation of the effect of castration on ADG can be very different, depending on the study design. In addition, Mach et al. (2009) evaluated bulls castrated with Burdizzo at 7 mo of age, a similar castration age to Knight et al. (1999) who castrated bulls with surgical castration; probably the different castration technique used would explain the difference in the numerical advantage on performance of bulls vs castrated animals between these two studys (6.25 vs 16.4 %). Morover, Field (1971) indicated that the detrimental effect of castration on growth rate and feed efficiency is more strongly expressed when animals are fed a higher plane of nutrition than when they are fed a lower plane of nutrition. In most

studies there is a lack of information regarding these factors, difficulting the comparison of the effect of castration on ADG between studies.

In the literature studies that evaluate the effect of immunocastration on performance have been conducted under very different vaccines types, vaccination programs and different breeds and feeding programs, so the impact of immunocastration on performance is not clear (Adams and Adams, 1992; Adams et al., 1996; Finnerty et al., 1994; Huxsoll et al., 1998; Cook et al., 2000; D'Occhio et al., 2001; Aïssat et al., 2002; Ribeiro et al., 2004; Hernández, et al., 2005; Amatayakul-Chantler et al., 2012). In some studies, the growth of immunocastrated animals is intermediate between the ADG of bulls and physically castrated animals. Aïssat et al. (2002) suggested that residual serum testosterone concentration (less than 1 ng/mL) observed in immunocastrated animals seem to be sufficient to improve the ADG compared to physically castrated animals.

2.6. Carcass and meat quality on castration

As indicated previously, castration is a common practice to reduce problems associated to sexual and aggressive behavior. Male behavior is associated to tissue damage in beef carcasses (carcass bruising) producing important economical losses. Castration reduces the number of mounts and the agonistic behavior, reducing carcass bruising problems (Mach et al., 2009). Moreover, it is possible that bulls, because of their temperament, may be stressed more easily than castrated animals and greater amounts of ante-mortem stress contribute to darker meat (Hedrick et al., 1969) and the increase of ultimate pH (Mc Veigh et al., 1982; Warriss, 1990; Purchas et al., 1992). However, many studies have shown that, in comparison with intact males, steers exhibit lower growth rates and feed efficiencies, dressing percentages and meat yields, but higher fatness and better organoleptic characteristics, particularly tenderness (Field, 1971).

Table 3. Effect of castration on long-term ADG depending on castration method, age, days on trial.

Author	Days on Trial	Method[a]	Age	Bulls	Physically castrated	Vaccinated
Champagne et al. 1969	182 d	-	Birth	1.23	1.04	
			2 mo		1.05	
			7 mo		1.01	
			9 mo		0.98	
Hedrick et al. 1969	585-570 d	-	-	0.86	0.71	
Martin and Stob, 1978	9.5 mo	-	3 mo	1.19	1.08	
Morgan et al. 1993	168 d	-	1 wk	1.40	1.08	
Huxsoll et al. 1998	150 d	-/I	4 mo	1.22	1.03	1.10
Knight et al. 1999	162 d	SUR	8 mo	0.71	0.58	
			17 mo		0.52	
Cook et al. 2000	84 d	I	9 mo	1.42		1.69
Keane et al. 2003	587-744 d	-	-	0.90	0.73	
Mach et al. 2009	121 d	BUR	7 mo	1.60	1.50	
Marti et al. 2011	140 d	RR	3 mo	1.51	1.37	

[a] Castration method: BAND= bands, BURD= Burdizzo, RR= rubber-ring, S= surgical, I = Immunocastration.

Tenderness has been identified as the main factor determining the consumer-eating satisfaction of beef (Jeleníková et al., 2008). A clear explanation of why beef form bulls is less tender than that from castrated animals is the lower proteolytic activity, slightly higher ultimate pH, lower levels of intramuscular fat, higher cooking losses, and possible a greater contribution of connective tissue components of meat from bulls compared with meat of steers (Purchas et al. 2002). Furthermore, as meat from castrated animals is tenderer than meat from intact males, meat from castrated animals needs less ageing to achieve an acceptalbe degree of tenderness (< 4 kg WBSF; Miller et al. 2001).

Carcasses of castrated animals are fatter than carcasses of intact males (Knight et al. 2000; Mach et al. 2009; Marti et al. 2011). Carcass fatness, as indicated by the subjective measurements of marbling and fat cover score and the objective measurements of the fat depth or intramuscular fat content, was consistently lower in bulls than in steers.

The lower fat content of bulls compared with castrated animals has been reported by other authors (Field 1971; Arthaud et al. 1977; Seideman et al. 1982), this suggests that castrated animals preferentially derived the energy into fat depots. Knight et al. (2000) suggested that age at castration could be used to manipulate carcass fatness. Castrating early-maturing cattle, such as Friesian (Barton & Pleasants 1997), at early ages may increase the carcass fatness and improve meat quality compared with castration at older ages.

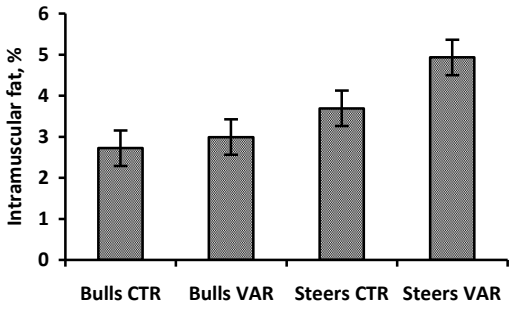
2.7. Alternatives to castration

No alternatives that simultaneously improve meat quality and sexual and aggressive behavior are so effective as castration.

There are some authors that tried to find alternatives to physical castration to reduce the performance losses, or improve meat quality without compromising welfare.

Vitamin A restriction has been proposed as a strategy to increase marbling in steers. Oka et al. (1992) showed that castrated animals consuming diets low in vitamin A had less serum retinol concentration and this was correlated with marbling. Gorocica-Buenfil et al. (2007) reported that vitamin A restriction during the finishing period may increase intramuscular fat without affecting subcutaneous fat deposition. In addition, this response to vitamin A restriction could be breed- and gender-dependent. In most of the studies where vitamin A restriction had no effect on marbling were conducted with Angus steers (Pyatt et al., 2005; Wang et al., 2007; Arnett et al., 2009). In contrast, when vitamin A restriction was evaluated in Holstein steers, increases in intramuscular fat were observed (Gorocica-Buenfil et al. 2007). When this strategy was evaluated in intact males, Marti et al. (2011) concluded that vitamin A restriction in bulls was lower (9.0% increase) than in castrated animals (33.6% increase), indicating that vitamin A restriction in bulls will have a poor impact and will not achieve similar intramuscular fat levels as those obtained with castration (Figure 6).

Figure 6. Effect of vitamin A restriction (CTR: control diet; VAR: vitamin A restriciton) in Holstein bulls and steers fed high-concentrate diets in i.m fat percentage (Marti et al. 2011).



3. LITERATURE CITED

Adams, T. E., and M. Adams. 1992. Feedlot performance of steers and bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 70:1691-1698.

Adams, T. E., C. A. Daley, B. M. Adams, and H. Sakurai. 1996. Testis function and feedlot performance of bulls actively immunized against gonadotropin-releasing hormone: effect of age at immunization. J. Anim. Sci. 74: 950-954.

Aïssat, D., J. M. Sosa, D. M. de Avila, K. P. Bertrand, and J. J. Reeves. 2002. Endocrine, growth, and carcass characteristics of bulls immunized against luteinizing hormone-releasing hormone fusion proteins. J. Anim. Sci. 80: 2209-2213.

Albright, J.L. 1983. Status of animal welfare awareness of producers and direction of animal welfare research in the future. J. Dairy Sci. 66:2208-2220.

Amann, R. P., and O. A. Walker. 1983. Changes in the pituitary-gonadal axis associated with puberty in Holstein bulls. J. Anim. Sci. 57: 433-442.

Amatayakul-Chantler, S., J. A. Jackson, J. Stegner, V. King, L. M. S. Rubio, R. Howard, E. Lopez, and J. Walker. 2012. Immunocastration of Bos indicus x Brown Swiss bulls in a feedlot with the gonadotropin-releasing hormone vaccine Bopriva® provides imporved performance and meat quality. J. Anim. Sci. published online June 4, 2012.

Arey, L. B. 1965. Developmental anatomy. A text book and laboratory manual embryology. WB Saunders Company, Phyladelphia.

Arnett, A. M., M. E. Dikeman, M. J. Daniel, K. C. Olson, J. Jaeger,and J. Perrett. 2009. Effects of vitamin A supplementation and weaning age on serum and liver retinol concentrations,carcass traits, and lipid composition in market beef cattle. Meat Sci. 81:596-606.

Arthaud, V. H., R. W. Mandigo, R. M. Koch, and A. W. Kotula. 1977. Carcass composition, quality and palatability attributes of bulls and steers fed different energy levels and killed at four ages. J. Anim. Sci. 44:53-64.

Bacha, F, N. Llanes, and E. Bueno. 2005. Alimentación de terneros en ausencia de promotores de crecimiento de tipo antibiótico: control de timpanismo y acidosis. XXI Curso de Especialización (FEDNA). 1: 132-158.

Barton, R. A., and A. B. Pleasants. 1997. Comparison ot the carcass characteristics of steers of different breeds and pre-weaning environment slaughtered at 30 month of age. New Zealand J. Agri. Res. 40:57-68.

Bass, J. J., A. J. Peterson, E. Payne, and M. P. Jarnet. 1977. The effect of neonatal estrogen treatment on plasma hormone levels and behaviour in pre- and post-pubertal bulls. Theriogenology. 8:59-71.

Berg, R. T. and R. M. Butterfield. 1968. Growth patterns of bovine muscle, fat and bone. J. Anim. Sci. 27:611-619.

Boesch, D., A. Steiner, L. Gygax, and M. Stauffacher. 2008. Burdizzo castration of calves less than 1-week old with and without local anaesthesia: Short-term behavioural responses and plasma cortisol levels. Appl. Anim. Behav. Sci. 114: 330-345.

Bretschneider, G. 2005. Effects of age and method of castration on performance and stress response of beef male cattle. A review. Livest. Prod. Sci. 97:89-100.

Broom, D. M. 1991. Animal welfare: concepts and measurement. J. Anim. Sci. 69: 4167-4175.

Cahill, V. R. 1964. Effects of sex differences in beef carcasses relative to cutability and palatability. Proc. Recip. Meat. Cont. 17:35.

Champagne, J. R., J. W. Carpenter, J. F. Hentges, Jr., A. Z. Palmer, and M. Koger. 1969. Feedlot performance and carcass characteristics of young bulls and steers castrated at four ages. J. Anim. Sci. 29:887-890.

Chase, C. C., Jr, R. E. Larsen, R. D. Randel, A. C. Hammond, and E. L. Adams. 1995. Plasma cortisol and white blood cell response in different breeds of bulls: comparison of two methods of castration. J. Anim. Sci. 73:975-980.

Comin, A., A. Prandi, T. Peric, M. Corazzin, S. Dovier, and S. Bovolenta. 2011. Hair cortisol levels of dairy cows from winter housing to summer highland grazing. Livest. Sci. 138:69-73.

Conner, J. G., P. D. Eckersall, M. Doherty, and T. A. Douglas, 1986. Acute phase response and mastitis in the cow. Res. Vet. Sci. 41:126-128.

Cook, R. B., J. D. Popp, J. P. Kastelic, S. Robbins, and R. Harland. 2000. The effects of active immunization against GnRH on testicular development, feedlot performance, and carcass characteristics of beef bulls. J. Anim. Sci. 78:2778-2783.

D'Occhio, M. J., W. J. Aspden, and T. E. Trigg. 2001. Sustained testicular atrophy in bulls actively immunized against GnRH: potential to control carcase characteristics. 66: 47-58.

Earley, B., and M. A. Crowe. 2002. Effects of ketoprofen alone or in combination with local anesthesia during the castration of bull calves on plasma cortisol, immunological, and inflammatory responses. J. Anim. Sci. 80:1044-1052.

Faulkner, D. B., T. Eurell, W. J. Tranquilli, R. S. Ott, M.W. Ohl, G. F. Cmarik, and G. Zinn. 1992. Perforamce and health of weanling bulls after butorphanol and xylazine administration at castration. J. Anim. Sci. 70: 2970-2974.

Field, R. A., 1971. Effect of castration on meat quality and quantity. J. Anim. Sci. 32:849-858.

Finnerty, M., W. J. Enright, C. A. Morrison, and J. F. Roche. 1994. Immunization of bull calves with GnRH analogue-human serum albumin conjugate: effect of conjugate dose, type of adjuvant and booster interval on immune, endocrine, testicular and growth responses. J. Reprod. Fertil. 101: 333-343.

Fisher, A. D., M. A. Crowe, M. E. Alonso de la Varga, and W. J. Enright. 1996. Effect of castration method and the provision of local anesthesia on plasma cortisol, scrotal circumference, growth, and feed intake of bull calves. J. Anim. Sci. 74:2336–2343.

Fisher, A. D., M. A. Crowe, E. M. O'Nauallán, M. L. Monaghan, D. J. Prendiville, P. O'Kiely, and W. J. Enright. 1997. Effects of suppressing cortisol following castration of bull calves on adrenocorticotropic hormone, in vitro interferon-gamma production, leukocytes, acute-phase proteins, growth, and feed intake. J. Anim. Sci. 75:1899-1908.

Fisher, A. D., T. W. Knight, G. P. Gosgrove, A. F. Death, C. B. Anderson, D. M. Duganzich, and L. R. Matthews. 2001. Effects of surgical or banding castration on stress responses and behaviour of bulls. Aust. Vet. J. 79: 279- 284.

Galbraith, H., D. G. Demspter, and T. B. Miller. 1978. A note on the effect of castration on the growth performance and concentration of some blood metabolites and hormones in British Friesian male cattle. Anim. Prod. 26:339-342.

González, L. A., K. S. Schwartzkopf-Genswein, N. A. Caulekett, E. Janzen, T. A. McAllister, E. Fierheller, A. L. Schaefer, D. B. Harley, J. M. Stookey, and S. Hendrick. 2010. Pain mitigation after band castration of beef calves and its effects on performance, behavior, Escherichia coli, and salivary cortisol. J. Anim. Sci. 88:802-810.

Gorocica-Buenfil, M.A., F.L. Fluharty, C.K. Reynolds, and S.C. Loerch. 2007. Effect of dietary vitamin a restriction on marbling and conjugated linoleic acid (CLA) content in Holstein steers. J. Anim. Sci. 85:2243-2255.

Gregory, K. E., and J. J. Ford. 1983. Effects of late castration, zeranol and breed group on growht, feed efficiency and carcass characteristics of late maturing bovine male. J. Anim. Sci. 56: 771-780.

Hedrick, H. B., G. B. Thompson and F. Krause. 1969. Comparison of feedlot performance and carcass characteristics of half-sib bulls, steers and heifers. J. Anim. Sci. 29:687-694.

Henricks, D.M. 1991. Biochemistry and physilogy of the gonadal hormones. Reproduction in domestic Animals. Academic Press, Inc. San Diego, C.A.

Hernández, J. A., E. L. Zanella, R. Bogden, D. M. de Avila, C. T. Gaskins, and J. J. Reeves. 2005. Reproductive characteristics of grass-fed, luteinizing hormone-releasing hormone-immunocastrated Bos indicus bulls. J. Anim. Sci. 83: 2901-2907.

Hirvonen, J. and S. Pyorala. 1998. Acute-phase response in dairy cowswith surgically-treated abdominal disorders. Vet. J. 155: 53–61.

Horadagoda, N.U., K.M.G. Knowx, H.A. Gibbs, S.W.J. Reid, A. Horadagoda, S.E.R. Edwards, P.D. Eckersall. 1999. Acute phase proteins in cattle: discrimination between acute and chronic inflammation. Vet Rec. 144: 437-441.

Huxsoll, C. C., E. O. Price, and T. E. Adams. 1998. Testis function, carcass traits, and aggressive behavior of beef bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 76: 1760-1766.

Imwalle, D. B., and K. K. Schillo. 2002. Castration increase pulsatile luteinizing hormone release, but fails to diminish mounting behavior in sexually experienced bulls. Dom. Anim. Endo. 22:223-235.

Jago, J. G., L. R. Matthews, J. J. Bass, and T. W. Knight. 1996. A comparison of two methods of castration of post-pubertal beef cattle and their effect on behavior, growth and ultimate pH. Proc. N.Z. Soc. Anim. Prod. 56:395-397.

Jago, J. G., N. R. Cox J. J. Bass, and L. R. Matthews, 1997. The effect of prepuertal immunization against gonadotropin-releasing hormone on the development of sexual and social behavior of bulls. J. Anim. Sci. 75:2609-2619.

Jeleníková, J., P. Pipek, and L. Staruch. 2008. The influence of ante-mortem treatment on relationship between pH and tenderness of beef. Meat Sci. 80:870-874.

Katz, L. S. 2007. Sexual behavior of domesticated ruminants. Horm. Behav. 52:56-63.

Keane, M. G. 1999. Effects of time of complete or split castration on performance of beef cattle. Ir. J. Agr Food Res. 38: 41-51.

Keane, M. G. 2003. Beef production from Holstein-Friesian bulls and steers of New Zealand and European/American descent, and Belgian Blue x Holstein-Friesian, slaughtered at two weights. Livest. Prod. Sci. 84:207-218.

Koren, L., O. Mokady, T. Karaskov, J. Klein, G. Koren, and E. Geffen. 2002.A novel method using hair for determining hormonal levels inWildlife. Anim. Behav. 63:403-406.

Knight, T. W., G. P. Cosgrove, A. F. Death, C.B. Anderson. 1999. Effect of interval from castration of bulls to slaughter on carcass characteristics and meat quality. New Zealand J. Agric. Res. 42:269-277.

Knight. T. W., G. P. Cosgrove, A. F. Death, C. B. Anderson, and A. D. Fisher. 2000. Effect of method of castrating bulls on their growth rate and liveweight. New Zealand J. Agric. Res. 43: 187: 192.

Lunstra, D. D., J. J. Ford, and S. E. Echternkamp. 1978. Puberty in beef bulls: hormone concentration, growth testicular development, sperm production and sexual aggressiveness in bulls of different breeds. J. Anim. Sci. 46:1054-1062.

Mach, N., A. Bach, A. Velarde, and M. Devant. 2008. Association between animal, transportation, slaughterhouse practices, and meat pH in beef. Meat Sci. 78:232-238.

Mach, N., A. Bach, C. E. Realini, M. Font i Furnols, A. Velarde, and M. Devant. 2009. Burdizzo pre-pubertal castration effects on performance, behavior, carcass characteristics, and meat quality of Holstein bulls fed high-concentrate diets. Meat Sci. 81:329-334.

Marti, S., C. E. Realini, A. Bach, M. Pérez-Juan, and M. Devant. 2011. Effect of vitamin A restriction on performance and meat quality in finishing Holstein bulls and steers. Meat Sci. 89: 412-418.

Martin, T. G., and M. Stob. 1978. Growth and carcass traits of Holstein steers, bulls and bulls implanted with Diethylstilbestrol. J. Dairy. Sci. 61:132-134.

McVeigh, J. M., P. V. Tarrant, and M. G. Harrington. 1982. Behavioral stress and skeletal muscle glycogen metabolism in young bulls. J. Anim. Sci. 54:790-795.

Mellor, D. J., V. Molony, I. S. Robertson. 1991. Effects of castration on behavior and plasma cortisol concentrations in young lambs, kids and calves. Res. Vet. Sci. 51:149-154.

Micol, D., M. P. Oury, B. Picard, J.F. Hocquette, M. Briand, R. Dumont, D. Egal, R. Jailler, H. Dubroeucq and J. Agabriel. 2009. Effect of age at castration on animal performance, muscle characteristics and meat quality traits in 26-month-old Charolais steers. Livest. Prod. Sci. 120: 116-126.

Miller, M., M. Carr, C. Ramsey, k. Crocket, and L. Hoover. 2001. Consumer thresholds for establishing the value of beef tenderness. J. Anim. Sci. 79:3062-3068.

Moberg, G. P. and J. A. Mench. 2000. The biology of animal stress: Basic principles and implications for animal welfare. CABI Publishing. London, UK.

Molony, V., K. E. Kent, and I. S. Robertson. 1995. Assessment of acute and chronic pain after differents methods of castration of calves. Appl. Anim. Behav. Sci. 46: 33-48.

Morgan, J. B., T. L. Wheeler, M. Koohmaraie, J. D. Crouse, and J. W. Savell. 1993. Effect of castration on myofibrillar protein turnover, endogenous proteinase activities, and muscle growth in bovine skeletal muscle. J. Anim. Sci. 71: 408-414.

Morton, D. B., and P. H. M. Griffiths. 1985. Guidelines on the recognition of pain, distress and discomfort in experimental animasl and an hypothesis for assessment. Vet. Rec. 116-431.

Murata, H., N. Shimada and M. Yoshioka. 2004. Current research on acute phase proteins in veterinary diagnosis: an overview. Vet. J. 168: 28-40.

Oka, A, T. Miki, Y. Yamasaki, T. Ariyoshi, and H. Fujii. 1992. Effects of vitamin A administration on meat quality of Japanese black cattle. J. Clin. Vet. Med. 10:2152.

Pang, W. Y., B. Earley. T. Sweeney, and M. A. Crowe. 2006. Effect of carprofen administration during banding or burdizzo castration of bulls on plasma cortisol, in vitro interferon-{gamma}production, acute-phase proteins, feed intake, and growth. J. Anim. Sci. 84: 351-359.

Pang, W. Y., B. Earley. V. Gath, and M. A. Crowe. 2008. Effect of banding or burdizzo castration on plasma testosterone, acute-phase proteins, scrotal circumference, growth, and helath of bulls. Livest. Sci. 117: 79-87.

Price, E., T. Adams, C. Huxsoll, and R. Borgwardt. 2003. Aggressive behavior is reduced in bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 81: 411-415.

Purchas, R. W., D. L. Burnham, and S. T. Morris. 2002. Effect of growth potential and growth path on tenderness of beef longissimus muscle from bulls and steers. J. Anim. Sci. 80:3211-3221.

Pyatt, N. A., L. L. Berger, D. B. Faulkner, P. M. Walker, and S. L. Rodriguez-Zas. 2005. Factors affecting carcass values and profitability in early-weaned Simmental steers: II. Days on feed endpoints and sorting strategies. J. Anim. Sci. 83:2926-2937.

Ribeiro, E. L. d. A., J. A. Hernandez, E. L. Zanella, M. Shimokomaki, S. H. Prudêncio-Ferreira, E. Youssef, H. J. S. S. Ribeiro, R. Bogden, and J. J. Reeves. 2004. Growth and carcass characteristics of pasture fed LHRH immunocastrated, castrated and intact Bos indicus bulls. Meat Sci. 68: 285-290.

Robertson, I. S., J. C. Wilson, and H. M. Fraser. 1979. Immunological castration in male cattle. Vet Res. 105:556-557.

Robertson, I. S., J. E. Kent, and V. Molony. 1994. Effect of different methods of castration on behaviour and plasma cortisol concentration in calves of three ages. Res. Vet. Sci. 56:8-17.

Rushen, J., A. M. de Passillé, M. A. G. von Keyserlingk and D. M. Weary. 2008. The welfare of cattle. Spinger. Dordreant, Holland.

Russell, E., G. Koren, M. Rieder, S. van Uum. 2012. Hair cortisol as a biological marker of chronic stress: Current status, future directions and unanswered questions. Psychoneuroendocrinology. 37: 589-601.

Sadlier, R. M. F. S. 1973. The Reporduction of Vertebrates. Academic Press, New York.

Seideman, S. C., H. R. Cross, R. R. Oltjen, B. D. Schanbacher. 1982. Utilization of the intact male for red meat production: a review. J. Anim. Sci. 55:826-840.

Stafford, K. J., D. J. Mellor, S. E. Todd, R. A. Bruce, and R. N. Ward. 2002. Effects of local anaesthesia or local anaesthesia plus a non-steroidal anti-inflammatory drug on the acute cortisol response of calves to five different methods of castration. Res. Vet. Sci. 73: 61-70.

Thüer, S., S. Mellema, M. G. Doherr, B. Wechsler, K. Nuss, and A. Steiner. 2007. Effect of local anaesthesia on short- and long-term pain induced by two bloodless castration methods of calves. Vet. J. 173: 333-342.a

Ting, S. T. L., B. Early, and M. A. Crowe. 2003a. Effect of repeated ketoprofen administration during surgical castration of bulls on cortisol, immunological function, feed intake, growt, and behavior. J. Anim. Sci. 81:1253-1264.

Ting, S. T. L., B. Early, J. M. L. Hughes, and M. A. Crowe. 2003b. Effect of ketoprofen, lidocaine local anesthesia, and combined xylazine during castration of beef cattle on stress responses, immunity, growth, and behavior. J. Anim. Sci. 81:1281-1293.

Turner, A. S. and C. W. McIlwraith. 1989. Techniques in large animal surgery. Lea and Febiger, Philadelphia.

Vanderwert, W., L. L. Berger, F. K. McKeith, R. D. Shanks, and P. J. Bechtel. 1985. Influence of zeranol implants on growth, carcass and palatability traits in bulls and late castrates. J. Anim. Sci. 61: 537-545.

van Tienhoven, A. 1983. Reproductive physilogy of vertebrates. Cornell University Press, New York.

Wang, W.J., S.P. Wang, Y.S. Gong, J.Q. Wang, and Z.L. Tan. 2007. Effects of vitamin A supplementation on growth performance, carcass characteristics and meat quality in Limosin x Luxi crossbreed steers fed a wheat straw-based diet. Meat. Sci. 77:450-458.

Warnock, T. M., T. A. Thrift, M. Irsik, M. J. Hersom, J. V. Yelich, T. D. Maddock, G. C. Lamb, and J. D. Arthington. 2012. Effect of castration technique on beef calf performance, feed efficiency, and inflammatroy response. J. Anim. Sci. 90:2345-2352.

Warris, P. D. 1990. The handling of cattle pre-slaughter and its effects on carcass and meat quality. App. Anim. Beh. Sci. 28:171-186.

Wolf, F. R., J. O. Almquist, and E. B., Hale. 1965. Prepuberal behaviour and puberal characteristics of beef bulls on high nutrient allowance. J. Anim. Sci. 24: 761-765.

Worrell, M. A., D. C. Clanton, and C. R. Calkins. 1987. Effect of weight at castration on steer performance on the feedlot. J. Anim. Sci. 64:343-347.

Zweiacher, E. R., R. M. Durham, B. D. Boren, and C. T. Gaskins. 1979. Effects of method and time of castration of feeder claves. J. Anim. Sci. 49: 5-9.

Chapter II

OBJECTIVES

The main objective of the present Thesis was to study the effect of physical castration and immunocastration in early-maturing bulls fed high-concentrates diets on welfare, performance, and carcass and meat quality. The specific objectives were:

1. To evaluate the effect of rubber-ring castration with anesthesia and analgesia in 3 month-old bulls on welfare indicators.

2. To find the optimum slaughter age of animals castrated at 3 month of age with rubber-rings and of animals castrated at 8 month of age with surgical castration, to achieve this goal technical data like performance and meat quality were necessary and helpful to take an economic decision, and increase the beef production output.

3. To evaluate the effect of immunocastration in 8 month-old bulls on welfare indicators, in order to evaluate if it was a good alternative from the welfare point of view to physical castration.

4. To evaluate the effect of immunocastration in 8 month old bulls on performance, carcass and meat quality, and to analyze if carcass and meat quality of immunocastrated animals was close to the carcass and meat quality of bulls physically castrated.

To achieve these specific strategies, four studies were conducted:

Study 1: Effects of ring castration with local anesthesia and analgesia in Holstein calves at three months of age on welfare indicators.

Study 2: Effect of castration and slaughter age on performance, carcass and meat quality traits of Holstein calves fed high-concentrate diets.

Study 3: Effect of gonadotropin-releasing hormone vaccine Bopriva® and band castration on beef cattle on welfare indicators.

Study 4: Effect of immunocastration of Holstein bulls fed high-energy diets with gonadotropin-releasing hormone vaccine Bopriva® on performance and meat quality.

Chapter III

EFFECTS OF RING CASTRATION WITH LOCAL ANESTHESIA AND ANALGESIA IN HOLSTEIN CALVES AT 3 MONTH OF AGE ON WELFARE INDICATORS

ABSTRACT

Forty-seven Holstein calves (130 ± 3.43 kg BW and 95 ± 1.5 d of age) were randomly assigned to 2 treatments (intact: INT, n = 23 or castrated: CAS, n=24) to evaluate the effect of ring castration at 3 mo of age on welfare indicators. Castration was performed with local anesthesia (lidocaine 2%, 3 mL in each testis and 2 mL in the scrotum) and analgesia (flunixin meglumine, i.m., 3 mg/kg BW). No local anesthesia and analgesia were used in INT calves. Serum cortisol concentration was determined at -120, 0, 30, 60, 90, and 180 min with respect to castration. At days 0, 1, 3, 7, 14, 21, 28, 35, 42, and 49, serum haptoglobin concentration was determined, rectal body and scrotal temperature were measured, lesions at the castration site were scored, and the activity and behavior of 18 calves (9 INT and 9 CAS) was recorded continuously during 24 h. Weekly BW and concentrate and straw DMI were recorded. To evaluate humoral immunity, 14 d after castration, ovalbumin was injected s.c. and serum antibody titers against ovalbumin before the injection and at day 35 were determined. At day 49 after castration, calves were i.v. injected with ACTH and at 0, 1, 2, and 4 h thereafter serum cortisol and testosterone concentrations were determined. Average daily gain was greater ($P < 0.001$) in INT than CAS calves (1.36 vs 1.16 ± 0.038 kg/d, respectively). Area under the curve of cortisol at castration day was reduced ($P < 0.05$) in CAS compared with INT calves (18 vs 33 ± 5.2 nmol/L/h, respectively). The main observed scrotal lesion scoring in CAS calves throughout the study was "0" corresponding to no swelling, inflammation or infection visible. However, scrotal lesion scoring classified as "1" (swelling) was greater ($P < 0.01$) 21 and 28 d after castration than at 1, 3, 7, and 14 d. Occurrence of abnormal standing was more frequent ($P < 0.001$) in CAS than in INT calves (2.6 vs 0.5 ± 0.03%, respectively) from 3 to 14 d after castration. Head turning tended ($P = 0.06$) to be greater

at day 14 of study in CAS than in INT calves (3.0 vs 2.6 ± 0.04%, respectively). At day 49, 100% of CAS calves had no testes and no serum testosterone was detected. In summary, ring castration of Holstein calves performed at 3 mo of age with local anesthesia and analgesia decreased ADG and affected some behavior traits during the first 14 d following castration. However, intake, serum cortisol and haptoglobin concentrations, rectal temperature and humoral immunity were not altered.

Key words: Beef, Castration, Welfare

1. INTRODUCTION

Castration of bulls has been proposed as a method to reduce meat quality problems of Holstein calves because it reduces sexual and aggressive behavior and improves carcass and meat quality (Mach et al., 2009). Ring castration at 3 mo of age requires less labor and rates of failure are lower compared with Burdizzo castration (Stafford, 2007). However, ring castration has been questioned from the welfare point of view (Molony et al., 1995; Thüer et al., 2007) based on an increased incidence of abnormal standing postures observed during short scanning periods in ring-castrated calves compared with intact calves. Stafford et al. (2002) evaluated the effect of local anesthesia or anesthesia plus a non-steroidal anti-inflammatory drug (NSAID) on acute serum cortisol in calves castrated using different castration methods. They reported that cortisol response was virtually eliminated when local anesthesia plus NSAID was administrated before castration, and thus, concluded that calves experienced little or no pain-induced distress during the 8-h period following castration. However, in that study no other welfare indicators were evaluated. Although animal welfare evaluation is complex and no standardized protocol exists, additional welfare indicators apart from behavior and plasma metabolites have been proposed (Broom, 1991). There is a need to establish objective parameters to quantify pain or stress caused by castration. The current study was aimed at assessing the impact of ring castration at 3 mo of age with local anesthesia and analgesia of Holstein calves on potential indicators of stress or pain. These indicators included performance (growth and intake), serum cortisol concentrations at castration day, serum haptoglobin concentration (tissue damage indicator), rectal and scrotal temperature, testes lesion scoring, humoral immunity, cortisol response following a ACTH challenge, and behavioral postures and activity during 7 wk following castration.

2. MATERIALS AND METHODS

2.1. Animals, Housing, and Diets

Forty-seven Holstein calves were used in a complete randomized design, and managed following the principles and guidelines of the IRTA Animal Care Committee (nº 4169). Animals were distributed in 47 individual partially-slated pens (1.20 x 1.45 m) allowing visual, olfactory and body contact with herd mates. Calves were weighed the day before castration (day -1), and stratified by full BW. Then, beginning with the heaviest and moving down the strata, animals were randomly assigned to one of 2 treatments: 23 calves remained intact (INT) and 24 calves were allocated to the castrated (CAS) treatment. Average initial BW and age of calves was 130 ± 3.4 kg and 95 ± 1.5 d (mean ± SE), respectively. The experiment was 7 wk in length. Ring castration was performed as described by Stafford et al. (2002). Calves assigned to the CAS group received a 3-mL injection of local anesthesia (lidocaine 2%, Xilocaina Ovejero, Laboratorios Ovejero, Spain), 20 min before castration, through the distal pole of each testicle. The testicles were then pushed dorsally off the needle and an additional 2 mL of local anesthesia was injected into the distal end of the scrotum. The scrotum was massaged to help diffusion of the local anesthetic. At the same time, 3 mg/kg BW of an analgesic (flunixin meglumine, Flunixin Inyectable Norbrook, Laboratorios Karizoo S.A., Spain) were administered i.m. Two rubber castration rings (Insvet, Huesca, Spain) were placed simultaneously on the neck of the scrotum just proximal to the testes using an elastrator (Insvet, Huesca, Spain). Two rings were used to ensure that castration would still occur if one broke. Calves assigned to the INT group were restrained during the same time as CAS calves to allow blood sample collection, but no local anesthesia or analgesia was applied. Calves were fed a concentrate (36.7% corn, 19.5% barley, 10.5% wheat

middlings, 10.1% corn gluten feed, 6.4% soy hulls, 5.0% wheat, 3.5% soybean meal, 2.5% canola, 1.5% calcium soaps of palm oil, 1.5% calcium carbonate, 0.80% urea, 0.70% sodium bicarbonate, 0.60% premix, 0.40% palm oil, 0.30% salt; 16.8% CP, 4.9% EE, 21.1% NDF, 6.0% ash, 0.9% Ca, 0.3% Cl, 0.3% Mg, 0.5% P, 0.7% K, and 0.4% Na; DM basis) and barley straw (3.5% CP, 1.6% EE, 70.9% NDF, and 6.1% ash; DM basis) in 2 separate troughs (0.3 m x 0.6 m x 1.2 m) until day 49 of experiment. Feeds were offered daily (feed weights were recorded) for ad libitum intake. Calves were housed at Cooperativa Agraria de Guissona experimental station (Guissona, Spain).

2.2. Measurements and Sample Collection

The day of castration is considered "d 0" of the study; all following references to the day of study are relative to day 0 when castration was conducted. On day 0, a 10-mL blood sample was harvested (without anti-coagulant additives; BD Vacutainer® Non-additive tube, BD Vacutainer®; Franklin Lakes, NJ) at -120, 0, 30, 60, 90, and 180 min relative to castration from CAS and INT calves by jugular venipuncture and subsequently analyzed for serum cortisol concentration. On days 0, 1, 3, 7, 14, 21, 28, 35, 42, and 49, a 10-mL blood sample was harvested by jugular venipuncture (BD Vacutainer® Non-additive tube) from all calves for subsequent serum haptoglobin analyses. All blood samples were centrifuged at 1500 x *g* at 4ºC for 15 min, and serum was decanted and stored at -20ºC until further analysis. Also, on days 0, 1, 3, 7, 14, 21, 28, 35, 42, and 49, rectal temperature using a digital electronic thermometer (Omron Healthcare BW, The Netherlands) and scrotal temperature at a horizontal distance of 20 cm from the testes using an infrared thermometer (Center® 350, Center Technology Corporation, Taiwan) were measured. On the same days, lesions at the castration site were scored following a 11-point scale described by Molony et al. (1995) with "0" indicating no swelling,

inflammation or infection visible; “0.5” to “2.0” depicting increasing degrees of swelling without obvious erythema; “2.5” and “3.0” corresponding to swelling with obvious erythema but without pus; and “3.5” to “5” indicating presence of pus with increasing inflammatory response. Time (in days) elapsed between castration and the fall of the testes was also individually recorded.

Behavior of 9 calves within each treatment was filmed continuously for 24 h on days 3, 7, 14, 21, 28, 35, 42, and 49 using a digital video-recording device (VDVR-9, Circontrol S. A., Terrassa, Spain) and digital color/monocromo cameras (VCAM-420DNA, Circontrol S. A.) fitted with heater resistors and autoiris vari-focal lenses (VLEN-2812VA, 2.8 to 11.5 mm, Circontrol S. A.) that were installed approximately 3 m above the ground. Each camera filmed simultaneously 2 pens. Videotapes were processed by scan-sampling every 10-min interval to represent behavior over an entire hour. Only 12 h of recordings (0800 to 2050) were used to create the scan sample data set, because the quality of the night recordings was not always acceptable. Behavioral categories were recorded (Table 1) and classified according to Molony et al. (1995) and Thüer et al. (2007) as active behaviors (no activity, eating, tail wagging, head turning, foot stamping, and sleeping) and postures (standing and lying). Animal BW and feed refusals were measured on days 7, 14, 21, 28, 35, 42, and 49.

Table 1. Description of postures and active behaviors of calves potentially affected by castration and recorded during the experiment.

Postures	
Normal standing	Standing eating , walking and playing with no obvious abnormality
Abnormal standing	Standing or walking with an obviously abnormal gait, e.g. swaying, standing stationary for more than 10-s period, continuous paddling or walking on knees
Normal lying	Ventral (sternal) recumbency with all legs folded under the body with head down or head up
Abnormal lying	Lying in ventral or lateral recumbency with full or partial extension of one or more legs with the head up or down
Active behavior	
No activity	Standing or lying with any described activity
Eating	Number of times a calf eating
Foot stamping	Hind limb lifted and forcefully placed on the ground or kicked against the abdomen while the animal was standing or lying
Tail wagging	Tail movement from side to side
Head turning	Movement of the head turned to a point on the body beyond the shoulder, e.g. scratching the testes and grooming
Sleeping	Number of times a calf sleeping or head down

On day 14, crystallized ovalbumin (GradeVII, Sigma Aldrich, St Louis, Missouri) was dissolved in sterile PBS (2mg/mL) and 4 mL of the final solution were injected subcutaneously in the midcervical regions of all animals. Immediately before the antigen injection at day 14, and at day 35 of the study, a 10-mL blood sample was harvested (BD Vacutainer® Non-additive tube) by jugular venipuncture to determine antibody titers against ovalbumin. At day 49, calves were intravenously injected with 2 IU porcine ACTH/kg $BW^{0.75}$ (Sigma-Aldrich, St Louis, MO, USA). Immediately before, and 1, 2, and 4 h after ACTH injection, a 10-mL blood sample was collected (BD Vacutainer® Non-additive tube) by jugular venipuncture for serum cortisol concentration analyses. Also on day 49 of study, an additional 10-mL blood sample was collected (BD Vacutainer® Non-additive tube) by jugular venipuncture for determination of serum

testosterone concentrations. All blood samples were centrifuged at 1500 x *g* at 4ºC for 15 min, and serum was decanted and stored at -20ºC until further analysis.

2.3. Chemical Analyses

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

Serum cortisol concentration was determined using an immunoassay technique (intra- and inter-assay CV of 5.6 and 7.1%, respectively; DRG-Cortisol ELISA EIA-1887, DRG Instruments, Germany). Haptoglobin was determined by hemoglobin binding method with the use of a commercial haptoglobin assay (intra- and inter-assay CV of 1.4 and 6.9%, respectively; Assay Phase Range, Tridelta Development Limited, Maynooth, Ireland).

Serum was analyzed for antibodies specific for ovalbumin by indirect ELISA using Maxisorp 96-microtiter plates (Nunc, Roskilde, Denmark) coated with 0.015 mg of ovalbumin per well. The plate was incubated for 18 h at 4ºC to allow ovalbumin to adhere to the wells. Following 18-h incubation, the plate was emptied and washed three times with 200µl PBS-0.05%Tween 20 (PBS-T) and further blocked with PBS-T for 2 h at 37 ºC. One hundred microliters of serum from samples obtained on days 14 and 35 were added to the plate at a dilution of 1/20 with PBS-T. This dilution was previously determined with a minimum of six different animals as the dilution giving the maximal signal. The plate with diluted serum was incubated for 1 h at 37 ºC and then washed 3 times with PBS-T. Horse Radish Peroxidase anti-bovine IgG (A3415 Sigma Aldrich) was

diluted 1:20000 with PBS-T and 100 µl were added to the wells for 1 h at 37 ºC. After 3 PBS-T washes, the HRP reaction was developed with 100 µl TMB substrate (Sigma Aldrich, St Louis, Missouri) and stopped with Stop Reagent for TMB substrate (Sigma Aldrich). Finally, the ELISA plate was read at 450 nm with a microplate reader (680 Bio-Rad, Hercules, CA, USA). All samples were analyzed in duplicate and the non-specific binding that occurred at day 14 was subtracted from the reading obtained at day 35 of study. Variations in readings among different ELISA plates were corrected by normalizing the readings from each sample within a plate to a reference control sample included in each plate. Intra- and inter-assay CV were 5.5 and 21.1%, respectively.

Serum concentrations of testosterone were determined using solid phase radioimmunoassay (intra- and inter-assay CV of 4.1 and 6.3%, respectively) following the manufacturer's instructions (Kit Coat-A-Count Total Testosterone Diagnostic Products Corporation, Los Angeles).

2.4. Calculations and Statistical Analyses

Area under the curve (AUC) of serum cortisol concentration was calculated for the first hours after castration or ACTH injection using the trapezoidal rule (Friend et al., 1977). Normality of the data prior to ANOVA analyses was evaluated the by frequency histogram distribution and the Shapiro-Wilk test. Serum cortisol and haptoglobin data were transformed to a log-scale to achieve a normal distribution prior any statistical analysis. Scan samples were multiplied by 10 and duration (per hour) of each behavior was converted to a percentage of the total time observed, and finally these percentages were transformed to a log-scale to achieve a normal distribution (Mitlohner et al., 2001). The values presented herein correspond to non-transformed means; however, SEM and *P*-values correspond to the ANOVA analyses using log-transformed data.

Performance, serum haptoglobin concentration, serum cortisol concentration data on castration day or after ACTH injection on day 49, body and scrotal temperature, and behavior data were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The mixed-effects model with repeated measures included initial BW, significant mean pretreatment serum values (-120 and 0 min on day 0) were used as covariates, castration, time (day or hour), and the interaction between castration and time, as fixed effects, and animal as a random effect. Time was considered a repeated factor, and for each analyzed variable, animal nested within treatment (the error term) was subjected to 3 variance-covariance structures: compound symmetry, autoregressive order one, and unstructured. The covariance structure that minimized Schwarz's Bayesian information criterion was considered the most desirable analysis. Cortisol AUC at days 0 and 49 were analyzed using the model described above but without accounting for the time effect (as there were no repeated measures). There were few wound and lesion scores that were ≤ 1 of the Molony et al. (1995) scale. Therefore, lesion scoring data were simplified into a binary classification: "0" indicating no swelling with visible inflammation or infection visible and "1" indicating presence of swelling with visible inflammation or infection. Scrotal lesion scores were conducted only on CAS calves (as INT calves had no wounds). Although the scoring took place throughout the study, only data referring to the first 28 d following castration were analyzed because after that time all testes had detached from the animals. Lesion scoring data, binary (0 or 1), were analyzed with the PROC GLIMMIX procedure of SAS (SAS Inst. Inc.). The mixed-effects model with repeated measures included time as a fixed effect and animal as random effect. For all analyses, significance was declared at $P \leq 0.05$ and tendencies were discussed at $0.05 < P \leq 0.10$.

3. RESULTS AND DISCUSSION

3.1. Intake and Animal Performance

Final BW (at day 49) and ADG (Table 2) were greater ($P < 0.001$) in INT (198 ± 1.6 kg, and 1.36 ± 0.038 kg/d, respectively) than in CAS calves (188 ± 1.6 kg, and 1.16 ± 0.038 kg/d, respectively). Body weight was affected ($P < 0.001$) by an interaction between castration and time. Up to day 7, no differences in BW between treatments were observed, but thereafter, CAS calves weighed less ($P < 0.001$) than INT calves. Average daily concentrate DMI ($P = 0.10$) and total DMI ($P = 0.09$) tended to be greater in INT than in CAS calves; however, when total DMI was expressed as % of BW, no differences were found. Gain to feed ratio was reduced ($P < 0.05$) in CAS compared with INT calves.

Table 2. Intake and performance of intact (INT) or ring-castrated Holstein calves at 3 mo of age with local anesthesia and analgesia (CAS).

	Treatment[1]			*P*-value[2]		
Item	INT	CAS	SEM	T	Time	T x Time
Initial and castration age, d	94	96	1.5	0.29	-	-
Initial BW, kg	130	130	3.4	0.90	-	-
Final BW (49 d of study), kg	198	188	1.6	0.001	-	-
ADG, kg/d	1.36	1.16	0.038	0.001	0.01	0.22
Concentrate DMI, kg of DM/d	4.5	4.3	0.09	0.10	0.001	0.47
Straw DMI, kg of DM/d	0.3	0.3	0.17	0.56	0.001	0.68
Total DMI, kg/d	4.8	4.6	0.09	0.09	0.001	0.77
Total DMI % of BW, kg of total DMI/ kg of BW	2.87	2.85	0.044	0.74	0.001	0.93
Gain to feed ratio	0.27	0.24	0.051	0.02	0.01	0.40

[1] INT = intact, CAS = calves ring-castrated at 3 mo of age with local anesthesia and analgesia
[2] T = treatment effect; Time = time effect (wk); T x Time = treatment by time interaction effect.

In the present study, differences in ADG between CAS and INT calves were observed from the first week after castration and maintained throughout the study. This result was unexpected, because in most studies (Fisher et al., 1996; Stafford et al., 2002; Ting et al., 2003; Pang et al., 2006), independently of age of castration, method of

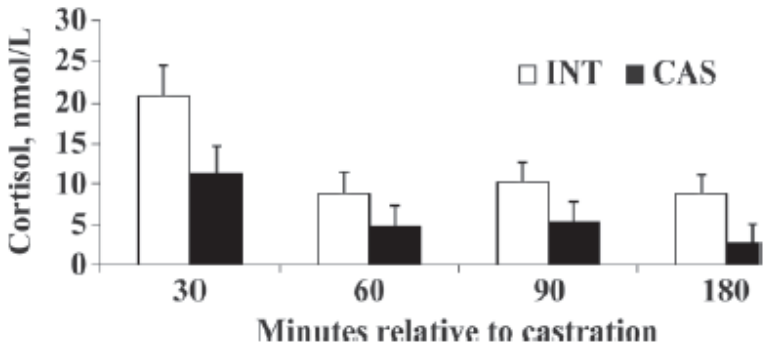
castration or local anesthesia/analgesia procedure used, ADG decreased within the first days after castration, as it occurred in the current study, but then ADG over the rest of the study lengths was unaffected. These differences could be attributed to the fact that in these cited studies, calves were fed grass silage supplemented with concentrate and mean ADG was lower than that observed in the present study. In agreement with the results of the current study Fisher et al. (2001), who reported ADG close to those obtained in the current study, observed that band- or surgically castrated calves at 9 or 14 mo of age grew less than intact calves. Morgan et al. (1993) observed that bulls had decreased muscle fractional degradation rates in contrast to castrated calves that could contribute to their greater growth availability. Fisher et al. (2001) administrated exogenous testosterone to castrated animals to investigate the roles of testosterone and castration in animal growth, but the exogenous testosterone administered was insufficient to increase plasma testosterone to the levels of intact calves and its effect on growth was minimal. Replacement of testosterone (using exogenous treatment) in castrated animals to levels equivalent to those of intact calves would help in the elucidation of the effects of the lack of testosterone in castrated animals on growth.

3.2. Serum cortisol concentration at castration day

Mean serum cortisol pretreatment values (-120 and 0 min before castration) were 16. 1± 3.9 and 18.2± 3.7 nmol/L for INT and CAS calves, respectively. As depicted in Figure 1, mean serum cortisol from 30 to 180 min after castration was reduced ($P < 0.001$) in CAS (5.6 ± 1.56 nmol/L) compared with INT calves (13.2 ± 1.56 nmol/L). In addition, AUC of serum cortisol concentration from 0 to 180 min relative to castration time tended ($P = 0.06$) to be greater in INT than in CAS calves (32 vs 19 ± 4.6 nmol/L/h, respectively). Serum cortisol concentration values observed in the present study were

close to those reported in other studies involving ring-castrated calves younger than 3 mo (Stafford et al., 2002; Thüer et al., 2007). Serum cortisol concentration at castration day has been proposed as an indicator of acute pain and stress. Local anesthesia locally inhibits action potentials in nerve cells by inhibiting sodium influx through the nerve cell membrane. Systemic administration of NSAID has been shown to act both centrally and peripherally, with central actions to be related to supraspinal effects causing inhibition of spinal transmission of nociceptive inputs (McCormarck, 1994).

Figure 1. Serum cortisol concentration (nmol/L) of intact (INT) or ring-castrated Holstein calves at 3 months of age with local anesthesia and analgesia (CAS).



In the current study, serum cortisol in the INT calves was greater than in CAS calves. These results are in disagreement with previous reports (Stafford et al., 2002; Thüer et al., 2007). The reason for such discrepancy is not clearly understood, but the low increase in serum cortisol in CAS calves could have been due to the administration of local anesthesia and analgesia. In addition, the castration method and the pain relief protocol (use of anaesthesia and/or analgesia and administration route and type of drug) affect the efficacy of acute pain alleviation (evaluated in the current study through serum cortisol concentrations). Stafford et al. (2002) concluded that injecting lidocaine into the testicles and into the distal end of the scrotum 20 min prior to the ring application successfully suppressed the acute pain and distress caused by ring castration as indicated by the elimination of an increase in serum cortisol. Furthermore, these authors considered

that giving ketoprofen in addition to a local anesthetic would be not necessary. Similarly, carprofen administered intravenously have been reported to only tending to reduce serum cortisol concentrations after band castration (Pang et al., 2006), but subcutaneous administration of carprofen in combination with epidural injection of lidocaine in surgically castrated animals reduced serum cortisol concentration more successfully than epidural-flunixin and epidural-alone treated calves (Stilwell et al., 2008). In contrast, Earley and Crowe (2002) and Ting et al. (2003) observed that systemic analgesia (ketoprofen i.v.) was more effective than local anesthesia or caudal epidural anesthesia at reducing cortisol response after castration in surgically or Burdizzo castrated calves, respectively.

3.3. Serum haptoglobin concentration

Increased production of acute phase proteins like haptoglobin aids in the regulation of inflammation following tissue damage (Baumann and Gauldie, 1994). Therefore, after castration, which causes local tissue trauma, an increase in serum haptoglobin could be expected. However, in the present study, castration did not result in increased serum haptoglobin concentration, with only a numerical increase in CAS animals being observed at days 3 and 7 after castration. Ting et al. (2005) studied the effect of Burdizzo castration performed at different ages on animal welfare and reported an increase in serum haptoglobin concentration the third day after castration in bulls castrated at 2.5 or 3.5 mo in contrast to bulls castrated at 1.5 mo of age. Pang et al. (2006) also observed an increase in serum haptoglobin concentration at the third day of castration in band-castrated bulls at 5.5 mo of age. However, as in the present study, when castration was performed with analgesia and carprofen (a NSAID), serum haptoglobin concentration was not different from that of intact bulls (Pang et al., 2006).

In addition, results from Ting et al. (2003) support the observation that NSAID administration (ketoprofen) at castration day mitigates the increase in serum haptoglobin after castration. As mentioned before, systemic administration of NSAID is pain-relieving and has anti-inflammatory effects.

3.4. Rectal temperature, scrotal temperature, scrotal lesion scoring

Castration did not affect rectal temperature (39.1 ± 0.032 ºC) throughout the study. Pang et al. (2006) only observed an increase in rectal temperature 2 d post-castration in band-castrated animals. In the present study, at day 35 relative to castration, testes started to slough off, and at day 49, all testes had completely sloughed off (55, 92, and 100% of CAS calves had no testes at 35, 42, and 49 d, respectively). Thus, scrotal temperature data were only analyzed from 0 to 28 d relative to castration. Mean scrotal temperature in CAS (28.8 ± 0.15 ºC) was reduced ($P < 0.001$) compared with INT (33.7 ± 0.15 ºC) calves. Ting et al. (2005) observed a decrease in the difference between rectal and scrotal skin temperature in Burdizzo-castrated bulls in contrast to intact bulls, because Burdizzo castration causes an important tissue damage and inflammation which raise scrotal temperature. In contrast, in the present study, the difference between rectal and scrotal temperatures in CAS animals (10.6 ± 0.21 ºC) was greater ($P < 0.001$) than in INT calves (5.7 ± 0.22 ºC) as ring castration ceases blood flow to the testes. In the present study, lesion scoring classified as "0", corresponding to no swelling, inflammation or infection visible, was the main recorded lesion. However, the prevalence of castration lesion scoring corresponding to inflammation "1" increased ($P < 0.05$) from values around 0 to 8% recorded from days 0 to 14 relative to castration, to a 33% on days 21 and 28. The low wound incidence was in agreement with serum haptoglobin concentration data observed in the present study, as castration did not affect serum haptoglobin. In contrast

to the present study, Molony et al. (1995) reported that ring castration of calves at 1 wk of age resulted in a mean lesion scoring around "4" 28 d after castration. In agreement with Molony et al. (1995), Thüer et al. (2007) observed an increased response to local palpation that persisted over 7 wk after castration in 1-mo-old ring-castrated animals independently of the use of local anesthesia (although no analgesia was used). In agreement with the current study, Stafford et al. (2002) reported that, independently of anesthesia or analgesia use, several days after castration the scrotum of calves castrated at 3 mo of age was dry with no swelling. In addition, these authors observed that at day 29 most animals had the scrotum shriveled and detached, and at day 38 most animals had small wounds.

3.5. Behavior

Normal standing, normal lying, and abnormal lying postures did not differ between treatments (Table 3).

Abnormal standing postures were greater from day 3 to day 14 after castration ($P < 0.01$) in CAS than in INT calves (Figure 2). Foot stamping was not observed in INT or CAS calves. Head turning tended ($P = 0.06$) to be greater 14 d after castration in CAS than in INT calves (Figure 3). Postures and active behavior 49 d after castration did not differ between CAS and INT calves (Table 3). The application of analgesia such as NSAID seems to be essential in avoiding abnormal behaviors after castration. In the two reference studies (Molony et al., 1995; Thüer et al., 2007), where abnormal behavior after ring castration was observed and was attributed to chronic pain, no analgesia was used. In support of the results from the current study, Ting et al. (2003) reported that in 13-mo-old Holstein bulls the use of an NSAID was more effective in reducing abnormal postures after Burdizzo-castration than the application of local anesthesia.

Table 3. Behavior of intact (INT) or ring-castrated Holstein calves at 3 months of age with local anesthesia and analgesia (CAS).

	Treatment[1]			*P*-value[2]		
Item[3]	INT	CAS	SEM[4]	T	Time	T x Time
Posture						
Normal standing, %	40.0	38.5	0.02	0.60	0.01	0.27
Abnormal standing, %	0.5	2.8	0.03	0.001	0.001	0.01
Normal lying, %	15.2	11.5	0.07	0.15	0.001	0.31
Abnormal lying, %	44.3	47.2	0.02	0.64	0.001	0.53
Active behavior						
Idle, %	67.1	65.6	0.01	0.48	0.06	0.85
Eating, %	16.3	15.0	0.02	0.32	0.001	0.91
Foot stamping, %	-	-	-	-	-	-
Tail wagging, %	6.8	7.0	0.05	0.80	0.001	0.97
Head turning, %	2.6	3.0	0.04	0.24	0.52	0.06
Sleeping, %	7.2	9.4	0.04	0.14	0.001	0.72

[1] INT = intact, CAS = calves ring-castrated at 3 mo of age with local anesthesia and analgesia
[2] T = treatment effect; Time = time effect (wk); T x Time = treatment by time interaction effect.
[3] Only data corresponding 12 h were selected (8:00 am to 20:50 pm) were used to create the scan sample data set. Behavior was analyzed at scan intervals of 10 min. To represent behavior over an entire hour, scan samples were multiplied by 10. Durations (per hour) of each behavior were converted to a percentage of the total time
[4] The values presented herein correspond to non-transformed means; however, SEM and P-values correspond to the ANOVA analyses using log-transformed data.

Figure 2. Evolution of abnormal standing posture (%) of intact (INT) or ring-castrated Holstein calves at 3 months of age with local anesthesia and analgesia (CAS) after castration.

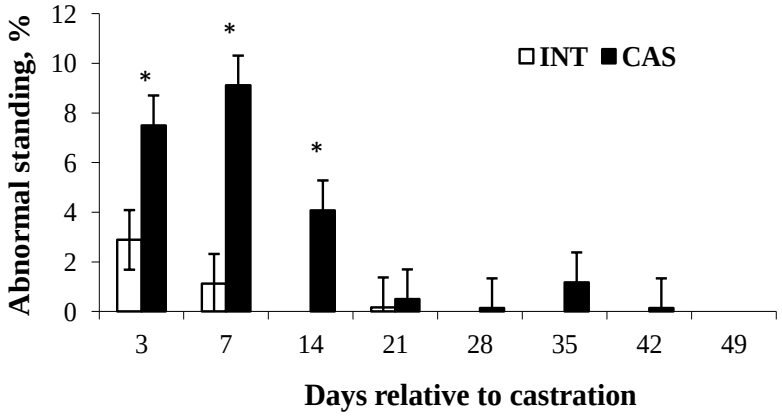
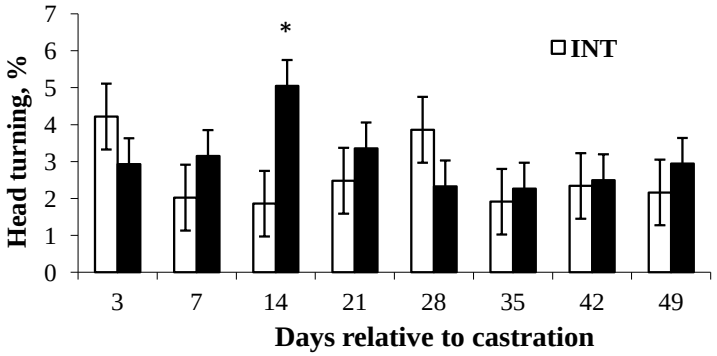


Figure 3. Evolution of head turning (%) of intact (INT) or ring-castrated Holstein calves at 3 mo of age with local anesthesia and analgesia (CAS) after castration.



3.6. Ovalbumin antibody titers and response to ACTH injection

Immunological assessment is a useful indicator of cattle welfare (Amadori et al., 1997). Castration did not affect humoral response against ovalbumin. The difference in ovalbumin immunoglobulins between ovalbumin vaccination (14 d) and at day 35 of the study were 0.62 ± 0.09 and 0.71 ± 0.09 antibody titers (A_{450}), for CAS and INT calves, respectively. Supporting the results presented herein, Pang et al. (2006) did not observe a detrimental effect of castration on cell-mediated immunity in the days following castration when evaluating different methods of castration and analgesia in 5-mo-old bulls. In contrast, Ting et al. (2003) observed a decrease in cell-mediated immunity 1 and 3 d after castration of 13-mo-old bulls using the Burdizzo method.

Castration did not affect serum cortisol response to ACTH injection. Serum cortisol AUC between 0 and 4 h after ACTH injection was 517 ± 32.9 and 486 ± 32.2 nmol/L/h for INT and CAS calves, respectively. The increase in plasma cortisol levels, as a consequence of the activation of the hypothalamic-pituitary-adrenal axis, is one of the best known and consistent neuroendrocrine responses to stress (Sevi et al., 2002). In welfare assessment of farm animals, the administration of exogenous ACTH is aimed to

stimulate adrenal secretion of cortisol, whose release may be strengthened by the existence of concurrent stressful event, and has been used to study the consequences of long-term stressors. Indeed, there is evidence that graded cortisol responses to stress can be attributed to both the relative stressfulness and the cumulative action of each stressor (Mears and Brown, 1997). Hence, in contrast to the results observed in the present study, it was expected that if castration had a long-term stressful effect, CAS calves would have a greater serum cortisol response to ACTH injection than INT calves. To our knowledge, there are no published studies that evaluate the effect of castration on cortisol response after ACTH injection.

3.7. Testosterone

Mean serum testosterone concentration at 49 d of study in INT calves was 269 ± 32 ng/dL, whereas in CAS calves no serum testosterone could be detected. Knight et al. (2000) observed that testosterone concentration decreased immediately at day 0 after band or surgical castration. Amann and Walker (1983) observed that serum testosterone levels decreased below 20 ng/dL 1 h after castration, whereas testosterone serum concentration of intact Holstein bulls around 22 wk of age was 267 ng/dL.

In summary, ring-castrated calves at 3 mo of age using analgesia and anesthesia had reduced growth, and during the first 14 d after castration increased abnormal standing, and tended to increase head turnings indicating distress during this period. Whether these transient alterations in behavior and reduced growth are sufficient to recommend avoiding castration at 3-mo of age using analgesia and anesthesia for welfare reasons should be further evaluated as no clear definition exists regarding how long these behavior traits should be altered to consider that castrated animals suffer chronic pain. On the other hand, the reduced growth observed in CAS bulls could be attributed to the

absence of serum testosterone as this hormone has anabolic effects.

4. IMPLICATIONS

Welfare indicators such as dry matter intake, serum cortisol, serum haptoglobin concentration, and wound healing are unaffected following ring castration performed with local anesthesia and analgesia at 3 mo of age. Despite growth is reduced and some behavior traits are altered during the first 2 wk after castration, ring-castrating calves at 3 mo using analgesia and anesthesia could be considered as a method that controls pain and does not greatly compromise animal welfare.

5. LITERATURE CITED

Amadori, M., I. L. Archetti, M. Bagni, E. Olzi, G. Caronna, and M. Lanteri. 1997. An immunological approach to the evaluation of Holstein Friesian cattle. J. Vet. Med. B. 44: 321-327.

Amann, R. P., and O. A. Walker. 1983. Changes in the pituitary-gonadal axis associated with puberty in Holstein bulls. J. Anim. Sci. 57: 433-442.

AOAC. 1995. Official Methods of Analysis. 16th ed. Assoc. Offic. Anal. Chem., Arlington, VA.

Baumann, H., and Gauldie, J. 1994. The acute phase response. Immunol. Today. 15: 74-80.

Broom, D. M. 1991. Animal welfare: concepts and measurement. J. Anim. Sci. 69: 4167-4175.

Earley, B., and Crowe. 2002. Effects of ketoprofen alone or in combination with local anesthesia during the castration of bull calves on plasma cortisol, immunological, and inflammatory responses. J. Anim. Sci. 80: 1044-1052.

Fisher, A. D., M. A. Crowe, M. E. Alonso de la Varga, and W. J. Enright. 1996. Effect of castration method and the provision of local anesthesia on plasma cortisol, scrotal circumference, growth, and feed intake of bull calves. J. Anim. Sci. 74: 2336-2343.

Fisher, A. D., T. W. Knight, G. P. Gosgrove, A. F. Death, C. B. Anderson, D. M. Duganzich, and L. R. Matthews. 2001. Effects of surgical or banding castration on stress responses and behaviour of bulls. Aust. Vet. J. 79: 279- 284.

Knight. T. W., G. P. Cosgrove, A. F. Death, C. B. Anderson, and A. D. Fisher. 2000. Effect of method of castrating bulls on their growth rate and liveweight. New Zeland J. Agric. Res. 43: 187-192.

Mach, N., A. Bach, C. Realini, M. Font-Furnols, A. Velarde, and M. Devant. 2009. Burdizzo pre-pubertal castration effects on performance, behaviour, carcass

characteristics, and meat quality of Holstein bulls fed high-concentrate diets. Meat Sci. 81: 329-334.

McCormack, K. 1994. Non-steroidal anti-inflammatory drugs and spinal nociceptive processing. Pain. 59: 9-43.

Mears, G. J., and F. A. Brown. 1997. Cortisol and β-endorphin responses to physical and psychological stressors in lambs. Can. J. Anim. Sci. 77:689–694.

Mitlohner, F. M., J. L. Morrow-Tesch, S. C. Wilson, J. W. Dailey, and J. J. Mc Glone. Behavioral sampling techniques for feedlot cattle. J. Anim. Sci. 79:1189-1193.

Molony, V., K. E. Kent, and I. S. Robertson. 1995. Assessment of acute and chronic pain after differents methods of castration of calves. Appl. Anim. Behav. Sci. 46: 33-48.

Morgan, J. B., T. L. Wheeler, M. Koohmaraie, J. D. Crouse, and J. W. Savell. 1993. Effect of castration on myofibrillar protein turnover, endogenous proteinase activities, and muscle growth in bovine skeletal muscle. J. Anim. Sci. 71: 408-414.

Pang, W. Y., B. Earley. T. Sweeney, and M. A. Crowe. 2006. Effect of carprofen administration during banding or burdizzo castration of bulls on plasma cortisol, in vitro interferon-{gamma}production, acute-phase proteins, feed intake, and growth. J. Anim. Sci. 84: 351-359.

Sevi, A., M. Albenzio, G. Annichchiarico, M. Caroprese, R. Marino, and L. Taibi. 2002. Effects of ventilation regimen on the welfare and performance of lactating ewes in summer. J. Anim. Sci. 80: 2349-2361.

Stafford, K. J., D. J. Mellor, S. E. Todd, R. A. Bruce, and R. N. Ward. 2002. Effects of local anaesthesia or local anaesthesia plus a non-steroidal anti-inflammatory drug on the acute cortisol response of calves to five different methods of castration. Res. Vet. Sci. 73: 61-70.

Stafford, K. J. 2007. Alleviating the pain caused by castration of cattle. Vet. J. 173: 245-247.

Stilwell, G., M. S. Lima, and D. M. Broom. 2008. Effects of nonsteroidal anti-inflammatory drugs on long-term pain in calves castrated by use of an external clamping technique following epidural anesthesia. Am. J. Vet. Res. 69: 744-750.

Thüer, S., S. Mellema, M. G. Doherr, B. Wechsler, K. Nuss, and A. Steiner. 2007. Effect of local anaesthesia on short- and long-term pain induced by two bloodless castration methods of calves. Vet. J. 173: 333-342.

Ting, S. T. L., B. Earley, J. M. L. Hughes, and M. A. Crowe. 2003. Effect of ketoprofen, lidocaine local anesthesia, and combined xylazine and lidocaine caudal epidural anesthesia during castration of beef cattle on stress responses, immunity, growth, and behavior. J. Anim. Sci. 81: 1281-1293.

Ting, S. T. L., B. Earley, I. Veissier, S. Gupta, and M. A. Crowe. 2005. Effects of age of Holstein-Friesian calves on plasma cortisol, acute-phase proteins, immunological function, scrotal measurements and growth in response to Burdizzo castration. Anim. Sci. 80: 377-386.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3588-3597.

Chapter IV

EFFECT OF CASTRATION AND SLAUGHTER AGE ON PERFORMANCE, CARCASS AND MEAT QUALITY TRAITS OF HOLSTEIN CALVES FED A HIGH-CONCENTRATE DIET

ABSTRACT

The aim of this study was to evaluate the effect of castration and slaughter age on performance and meat quality of Holstein bulls fed a high-concentrate diet. A total of 132 animals (116 ± 3.7 kg of BW and 97 ± 2.4 d of age) were randomly allocated in 6 pens following a 3 x 3 factorial arrangement of treatments. Three castration ages (bulls, castration at 3 mo: CAS3, and castration at 8 mo of age: CAS8) and 3 slaughter ages (10, 12, and 14 mo of age) were evaluated. Feed intake was recorded daily using a computerized concentrate feeder, and BW was recorded every 14 d. The 9 to 11th rib section was removed at 24 h post-mortem and dissected into lean, fat and bone, and meat quality was evaluated on the LM. Castration, at 3 or 8 mo of age, reduced ($P < 0.001$) ADG and muscle pH, and impaired ($P < 0.01$) feed efficiency. As slaughter age increased, concentrate consumption increased linearly ($P < 0.001$), and feed efficiency was reduced linearly ($P < 0.001$). Slaughter age also affected ($P < 0.001$) meat pH. Significant interactions between castration and slaughter ages were also observed in carcass conformation ($P < 0.05$), fatness ($P < 0.001$), and percentage of subcutaneous fat ($P < 0.01$), carcass dressing percentage ($P < 0.05$), intramuscular fat ($P < 0.05$), and tended to be significant in intermuscular fat ($P = 0.09$). In Holstein animals, castration age affects performance and meat pH regardless of slaughter age, and slaughter age affects performance and meat pH independently of castration. However, in Holstein animals, castration affects several characteristics related to fat deposition differently depending on slaughter age, such as carcass fat cover, and intramuscular, intermuscular, and subcutaneous fat.

Key words: beef, castration, meat quality

1. INTRODUCTION

Optimum slaughter age to obtain maximum net return and desired meat quality may differ depending on castration age, gender, nutrition, and genetics, together with economic factors such as feed costs and carcass prices (Mark et al., 2000; Pyatt et al., 2005). Knight et al. (1999a) studied different castration and slaughter ages in grazing crossbred beef animals, and proposed post-pubertal castration of calves (13 mo of age) followed by a finishing period as an effective management strategy to maximize benefits. This proposed strategy maintains the performance advantages of intact males until 13 mo and the benefits of castration on meat quality characteristics thereafter. In Holstein bulls slaughtered at 12 mo of age, pre-pubertal ring castration was recently proposed (Marti et al., 2010) as a castration procedure to reduce labor and rates of failure compared with those obtained using Burdizzo castration (Mach et al., 2009). However, ring castration at 3 mo of age reduced feed efficiency and carcass weight compared with bulls (Marti et al., 2011**)** and post-pubertal (8 mo of age) castrated animals (Mach et al., 2009).

In the recent years, in Europe feed prices have drastically increased and as a consequence production costs have risen and net returns have decreased. Amer et al. (1994) observed that in some breeds, the reduction of slaughter age could be an alternative to maximize net return. Therefore, reducing slaughter age of these pre-pubertal castrated steers could be an alternative to improve feed efficiency without compromising carcass and meat quality. The aim of this study was to provide the necessary understanding about the effects of age of castration and age of slaughter on performance, carcass and meat quality of Holstein bulls and steers fed high-concentrate diets to determine the optimum castration age and its corresponding optimum slaughter.

2. MATERIALS AND METHODS

2.1. Animals, Housing, and Diets

One hundred and thirty-two weaned Holstein calves (116 ± 3.7 kg of BW and 97 ± 2.4 d of age) were managed following the principles and guidelines of the Animal Care Committee of IRTA and randomly distributed to one of the 9 treatments following a complete randomized design with a 3 x 3 factorial arrangement of treatments: bulls, castrated animals at 3 mo of age (CAS3), and animals castrated at 8 mo of age (CAS8), slaughtered at 10, 12, and 14 mo of age. Number of replicates for each treatment was 14 or 15. Animals were housed at a commercial farm (Montgai, Spain) in 6 pens (2 pens for each castration age). Animals in the CAS3 group were castrated using ring castration as described elsewhere (Marti et al., 2010), whereas CAS8 animals were surgically castrated following Ting et al. (2003a). In each pen, animals had access to one computerized concentrate feeder (GEA SurgeWestfalia, Germany) that recorded individual daily concentrate consumption (Devant et al., 2012), to one water source, and also ad libitum access to barley straw (3.5% CP, 1.6% EE, 70.9% NDF, and 6.1% ash, 1.45 Mcal EM/kg; DM basis) in a separate feed trough (3 m x 1.12 m x 0.65 m; 7 feeding spaces). The amount of straw offered to each pen was recorded to estimate the total amount of straw consumed; however as straw was also used for bedding, these data are only estimates. In the present study, apparent straw intake was around 756 ± 58 g/d (Devant et al., 2012), corresponding to a concentrate to straw ratio of 89 to 11. All animals were fed ad libitum the same concentrate (40% corn, 21% barley, 15% wheat middlings, 14.3% soybean meal, 5% soyhulls, 2.6% palm oil, 1.6% calcium carbonate, 0.3% salt, 0.2% premix; 14.6% CP, 5.4% EE, 16.7% NDF, 4.6% ash, 3.25 Mcal EM/kg, 0.7% Ca, 0.4% P, 0.4%

Cl, 0.1% Na; DM basis) throughout the study. Body weight was recorded every 14 d until animals were transported to the slaughterhouse.

2.2. Chemical Analyses

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

2.3. Carcass and Meat Quality Measurements

At 10, 12 and 14 mo of age, animals were randomly selected and transported to a commercial slaughterhouse (Mercabarna, Barcelona, Spain). Animals from different treatments were not mixed in the truck, and the transport distance was less than 150 km. Animals were stunned using a captive-bolt pistol and dressed according to commercial practices. The HCW (with tail attached and without kidney, liver and heart) was recorded, and the degree of carcass fatness and conformation were graded according to the (S) EUROP categories (EU Regulation No. 1208/81, 1026/91) and into EU classification system into 1.2.3.4.5 (EU Regulation No. 1208/81), respectively. The conformation class designated by the letter "E" (excellent) describes carcasses with all profiles convex to super-convex, and with exceptional muscle development, whereas the conformation classified as "U" (very good) present profiles on the whole straight, and good muscle development. Carcasses classified as "R" (good) present profiles on the whole straight, and good muscle development. Carcasses classified as "O" (fair) presents profiles straight to concave, and with average muscle development, whilst carcasses classified as "P" (poor) present all profiles concave to very concave with poor muscle development. In addition, the degree of fat cover describes the amount of fat on the outside of the carcass

and in the thoracic cavity. The class of fat cover from 1 to 5, classifies 1 (low) describes none to low fat cover, whereas the class 5 (very high) describes an entire carcass covered. After 24 h of carcass chilling, a bone-in rib section between the 9th and 11th ribs removed as outlined by Hankins and Howe (1946) and used to determine physical separable fat, lean, and bone, and to predict carcass composition using the equations proposed by Hankins and Howe (1946). In addition, fat was separated into subcutaneous fat (s.c.), intermuscular fat, and internal fat (flare fat); and lean was separated into LM and remaining lean without LM as based on Walstra and Merkus (1995).

Muscle pH was measured at 24 h *post-mortem* using a portable pH-meter (PH 25 DL, Crison, Alella, Spain) equipped with a xerolyt electrode inserted in the LM at the 11th rib level. The LM was removed from each rib section, cut between the 10th and 11th rib and instrumental color measurements recorded. Lightness (L^*), redness (a^*), and yellowness (b) were measured on the exposed cut surface of the LM after 30 min of bloom time using a Minolta colorimeter (CR-400, Minolta Inc., Osaka, Japan) in the CIE-LAB space (Commission International de l'E'clairage, 1976) with illuminant D65 and 2º viewing angle. After measuring color, the LM was cut into 4 steaks (2.5 cm each) which were individually vacuum-packaged, two of them were immediately frozen (day 0) and the other two were stored at 4ºC during 7 d of aging and then frozen for subsequent sensory analysis and Warner-Bratzler shear force (WBSF) measurements. The remaining steak of the LM was vacuum-packaged and stored at -20ºC until determination of intramuscular (i.m.) fat content as described in Marti et al. (2011), protein and humidity using near infrared transmission (FoodScan^{TM} analyzer, Type 78800, FOSS, Hilleroed, Denmark).

The steaks for WBSF analysis were thawed for 24 h at 2˚C, wrapped in aluminum foil and cooked to an internal temperature of 71ºC in an oven pre-heated to 200ºC. Sample internal temperature was monitored with a data logger and a thermocouple probe inserted

horizontally at the steak midpoint. Cooked steaks were allowed to come to room temperature during 2 h before 6 cores (1 cm^2 cross-section x 3 cm long) were removed per steak with the fiber direction parallel to the longest dimension of the sample, and shared perpendicular to the direction of the blade. The WBSF was measured using a texture analyzer Alliance RT/5 (MTS Systems Corp., Eden Prairie, MN, USA) equipped with a Warner-Bratzler blade with crosshead speed set at 2 mm/s.

For sensory attributes evaluation, thawing and cooking were accomplished using the same protocol described previously for WBSF determination. After cooking, each sample was cut into subsamples. Each subsample was immediately wrapped in aluminum foil, codified and kept in a heater to maintain a constant temperature of 60ºC until panelist assessment (Serra et al., 2008). Trained panelists evaluated the cooked subsamples in individual booths provided with red light. The subsamples were tasted in a different order in each session to eliminate carry-over effects (MacFie and Thompson, 1988). Panelists were required to rate each subsample for beef flavor, initial hardness, overall hardness, and juiciness. Each attribute was rated on a non-structured 10-point scale, with score 0 equivalents representing the least and 10 the greatest intensity of the attribute.

2.4. Statistical analyses

Animal was the experimental unit. Normally distributed variables (performance and meat quality data) were analyzed using a mixed-effects model (SAS Inst. Inc., Cary, NC) including castration age, slaughter age, and the interactions between these factors, as fixed effects, and pen as a random effect. The model was also tested for linear and quadratic effects of slaughter age and their interaction with the other fixed effects. To analyze sensory evaluation data, the same model was used and also included panelist and session as random effects was used. Carcass conformation and fatness were analyzed

using the Chi-square test of SAS (SAS Int. Inc., Cary, NC). Significance was established at $P < 0.05$, and trends at $P \leq 0.10$.

3. RESULTS

Four bulls and three CAS8 animals were removed from the study due to health problems unrelated to treatments (pneumonia, anorexia, and lameness), and their corresponding data were excluded from all analyses. Also, all data from one CAS8 animal that died the day after castration (the necropsy did not lead to a clear diagnosis) were excluded as well.

To simplify the presentation of results, tables herein show least squares means for the main effects, because only a few interactions between castration and slaughter age were significant ($P > 0.05$) and these are indicated in the tables with least square means being described in the text.

3.1. Performance

No interactions between castration age and slaughter age were found for performance data. The final BW of bulls was greater ($P < 0.001$) than that of CAS8 and CAS3. No differences in final BW were observed between CAS8 and CAS3. Final BW increased linearly ($P < 0.001$) with slaughter age; final BW of 14 mo was 16.0 and 27.7**%** greater than final BW at 12 and 10 mo of age, respectively (Table 1). Average daily gain was not affected by slaughter age; however, castration had a detrimental effect ($P < 0.001$) on ADG. Bulls had a greater ADG than CAS8 and CAS3; and CAS8 tended ($P = 0.10$) to have a greater ADG than CAS3 (Table 1).

Table 1. Intake and performance of Holstein bulls, bulls castrated at 8 mo of age (CAS8) or at 3 mo of age (CAS3) and slaughtered at 10, 12 and 14 mo of age fed a high-concentrate diet

	Treatment								
	Castration age[1]			Slaughter age[2]				*P-value*[3]	
Item[4]	Bulls	CAS8	CAS3	10	12	14	SEM	CA	SA
Initial age, d	98	98	95	98	95	97	1.4	0.25	0.32
Final age, d	354	354	351	298^{c}	353^{b}	410^{a}	1.4	0.26	< 0.001
Initial BW, kg	116	117	114	118	113	115	2.2	0.79	0.29
Final BW[4], kg	489^{a}	470^{b}	459^{b}	397^{c}	473^{b}	549^{a}	5.2	< 0.001	< 0.001
ADG, kg/d	1.47^{a}	1.41^{b}	1.36^{b}	1.43	1.40	1.41	0.019	< 0.001	0.62
Concentrate DMI[4], kg/d	6.81	6.90	6.63	6.19^{c}	6.78^{b}	7.37^{a}	0.09	0.13	< 0.001
Feed efficiency[5]	0.22^{a}	0.21^{b}	0.21^{b}	0.23^{a}	0.21^{b}	0.19^{c}	0.002	< 0.01	< 0.001

[1] Bulls, CAS3 = bulls castrated at 3 mo of age, CAS8= bulls castrated at 8 mo of age.
[2] 10= animals slaughtered at 10 mo of age, 12= animals slaughtered at 12 mo of age, 14= animals slaughtered at 14 mo of age.
[3] CA= effect of castration age; SA = effect of slaughter age.
[4] *P*-value corresponding to the linear (L) effect ($P < 0.001$); and to the quadratic (Q) effect ($P = 0.96$) of slaughter age.
[5] *P*-value corresponding to the linear (L) effect ($P < 0.001$); and to the quadratic (Q) effect ($P = 0.29$) of slaughter age

No differences were observed in concentrate intake between bulls and castrated animals (Table 1), the same results were obtained when concentrate intake or nutrient intake were expressed as percentage of metabolic BW (data not shown). As a result of increased ADG in bulls, these animals were more efficient ($P < 0.01$) than castrated ones. Concentrate intake increased linearly ($P < 0.001$) with slaughter age, with animals slaughtered at 10 mo consuming 9.5% less than animals slaughtered at 12 mo, and animals slaughtered at 12 mo consuming 8.9% less than animals slaughtered at 14 mo of age. A similar slaughter age effect was observed when concentrate intake and nutrient intake were expressed as percentage of metabolic BW (data not shown). Concentrate intake expressed as percentage of BW increased linearly ($P < 0.001$) with slaughter age (9.6, 10.6, 11.5 % ± 1.4 of concentrate intake relative to metabolic BW, for 10, 12 and 14

mo of slaughter age, respectively). Because slaughter age did not affect ADG but did affect concentrate intake, feed efficiency decreased linearly ($P < 0.001$) with slaughter age.

3.2. Carcass quality

Hot carcass weight was affected by castration age ($P < 0.001$) and describing a linear increase ($P < 0.001$) with slaughter age. Hot carcass weight of bulls was greater ($P < 0.01$) than that of castrated animals (Table 2). The HCW of animals slaughtered at 14 mo of age was 16**%** greater ($P < 0.001$) than that of animals slaughtered at 12 mo of age, and HCW of the latter was 19.9**%** greater than that of animals slaughtered at 10 mo of age. In dressing percentage the interaction between castration age x quadratic slaughter age tended to be significant with a nonlinear effect ($P = 0.06$). When animals were slaughtered at 10 mo of age, dressing percentage was greater ($P < 0.01$) in bulls (52.8 ± 0.34 %) than in CAS8 (51.1 ± 0.34 %), and tended to be greater ($P = 0.07$) in bulls than in CAS3 (51.9 ± 0.34%); however, no differences among castration ages (CAS8 and CAS3) were observed. At 12 mo of age, the lowest dressing percentage ($P < 0.01$) was observed in CAS8 (52.1 ± 0.34 %), and no differences ($P = 0.13$) in dressing percentage were observed between bulls (53.9 ± 0.34 %) and CAS3 animals (53.2 ± 0.34 %). Last, at 14 mo of age no differences in dressing percentages among treatments were observed (52.9, 52.7, 52.5 ± 0.34 %, for bulls, CAS8, and CAS3, respectively).

Table 2. Carcass quality of Holstein bulls, bulls castrated at 8 mo of age (CAS8) or at 3 mo of age (CAS3) and slaughtered at 10, 12 and 14 mo of age fed a high-concentrate diet

	Treatment								
	Castration age[1]			Slaughter age[2]				*P-value*[3]	
Item[6]	Bulls	CAS8	CAS3	10	12	14	SEM	CA	SA
BW before slaughter[4], kg	494[a]	478[b]	464[b]	405[c]	475[b]	555[a]	5.5	< 0.001	< 0.001
Hot carcass weight[4], kg	262[a]	249[b]	244[b]	210[c]	252[b]	293[a]	3.2	< 0.001	<0.05
Dressing percentage[5, 6], %	53.2[a]	52.0[c]	52.5[b]	51.9[b]	53.0[a]	52.7[a]	0.20	< 0.001	< 0.001
Carcass fat cover[5], %									
1	15.0	2.5	0	17.9	0	0			
2	57.5	50.0	47.3	79.5	66.7	11.6		< 0.01	<0.001
3	27.5	47.5	52.7	2.6	33.3	88.4			
Carcass conformation[5], %									
P	5.0	17.5	13.6	23.1	11.9	2.3			
O	95.0	80	86.4	76.9	88.1	95.3		0.25	< 0.01
R	0	2.5	0	0	0	2.33			

[1] Bulls, CAS3 = bulls castrated at 3 mo of age, CAS8= bulls castrated at 8 mo of age.
[2] 10= animals slaughtered at 10 mo of age, 12= animals slaughtered at 12 mo of age, 14= animals slaughtered at 14 mo of age.
[3] CA= effect of castration age; SA = effect of slaughter age
[4] L $P < 0.001$; Q $P > 0.10$
[5] Interaction between castration age and slaughter age (dressing percentage, $P < 0.05$; carcass fat cover, $P < 0.001$; carcass conformation, $P < 0.05$; separable subcutaneous fat, $P < 0.05$; separable intermuscular fat, $P = 0.07$)

Carcass fat cover and conformation were affected by an interaction ($P < 0.05$) between castration and slaughter age. Animals slaughtered at 10 mo of age presented 91.7, 53.8 and 85.7 % of carcass classified as "O" for bulls, CAS8 and CAS3, respectively; these proportions increased with slaughter age in all animals; however, the increase was more pronounced for CAS8 animals. At 12 mo of slaughter age, 92.9, 92.3 and 80 % of carcasses were classified as "O" for bulls, CAS8 and CAS3, respectively. At 14 mo of slaughter age, 100, 92.9 and 93.3 % of carcasses were classified as "O" for bulls, CAS8 and CAS3, respectively. At 10 mo of age, CAS8 animals had the greatest

percentage of carcasses classified as "P" (the lowest carcass classification) compared with bulls and CAS3 animals (42.6 %, 8.3 %, 14.3 % for CAS8, bulls, and CAS3 animals, respectively). However, at 12 mo of age, CAS8 animals reduced the percentage of carcasses classifies as "P" to 7.7 % while CAS3 animals increased this percentage to 20%. At 14 mo of age, only CAS3 animals registered carcasses classifies as "P" (6.7 %). However, carcasses classified as "R", which corresponds to the best carcass conformation registered in Holstein animals under the production system described herein, were only registered at 14 mo of age in CAS8 with a percentage of 7.1 %. At 10 mo of age, 50 % of bulls and above 90% of castrated animals (CAS3 and CAS8) slaughtered were classified as "2" for carcass fat cover, and 50% of bulls and 7.7 % of CAS8 were classified as "1" (the lowest carcass fat cover classification). At 12 mo of age and 14 mo of age no carcasses classified as "1" were recorded. At 12 mo of age the carcass fat cover of bulls increased, and 100% of bull carcasses were classified as "2". For CAS8 animals, no carcasses were classified as "3" at 10 mo of slaughter age, and at 12 mo of age this proportion of carcasses classified as "3" was 38.5 %. Animals castrated at 3 mo of age had 7.2 % of carcass classified as "3" at 10 mo of age and this proportion increased when CAS3 were slaughtered at 12 mo of slaughter age (60 %). At 14 mo of slaughter age, 78.6% of bulls, 100 % of CAS8 and 86.7 % of CAS3 were classified as "3" of carcass fat cover.

3.3. Rib section data

Castration age had no effect on section weight of the 9-10-11th ribs (Table 3). However, rib weight increased linearly ($P < 0.001$) with slaughter age (Table 3). Bulls had 31.5% less ($P < 0.001$) proportion of rib-separable fat than CAS3, and the proportion of separable fat of CAS8 was 8.9% less ($P < 0.001$) than that of CAS3. The proportion of

rib-separable fat showed a quadratic effect ($P < 0.001$) with slaughter age; between 10 and 12 mo of age rib-separable fat increased ($P < 0.001$), and from 12 to 14 mo of age no further increase in the proportion of rib-separable fat was observed. A non-linear slaughter age by castration age interaction ($P < 0.01$) was observed in the proportion of rib-separable s.c. fat. At 10 mo of age, the proportion of rib-separable s.c. fat tended ($P = 0.08$) to be greater in CAS3 (7.6 ± 0.76 %) compared with CAS8 (5.7 ± 0.76 %) and bulls (3.8 ± 0.76 %). At 12 mo of age, this proportion was also greater ($P < 0.05$) in CAS3 (13.2 ± 0.76 %) than CAS8 (10.9 ± 0.76%) and bulls (8.3 ± 0.76 %). From 12 to 14 mo of age a pronounced decrease ($P < 0.01$) in the proportion of rib-separable s.c. fat was observed in CAS3 animals, and at 14 mo of age this proportion did not differ among bulls (7.0 ± 0.76 %), CAS8 (9.7 ± 0.76 %), and CAS3 (7.5 ± 0.76 %). In the proportion of rib-separable intermuscular fat a non-linear slaughter age by castration age interaction tended to be significant ($P = 0.09$). The proportion of rib-separable intermuscular fat tended ($P = 0.10$) to decrease from 10 (8.8 ± 0.69 %) to 12 (7.4 ± 0.69 %) mo of age in CAS8, whereas in bulls (7.2 and 7.4, ± 0.69%, for 10 and 12 mo of age, respectively) and CAS3 (10.9 and 10.4, ± 0.69 %, for 10 and 12 mo of age, respectively) did not change. From 12 to 14 mo of slaughter age this proportion increased in all treatments, with CAS3 showing the greatest ($P < 0.05$) rib-separable intermuscular fat (16.5 ± 0.69 %) compared with bulls (10.1 ± 0.69 %) and CAS8 (13.9 ± 0.69 %).

Castration age affected the proportion of total separable lean ($P < 0.01$). Bulls had a 16.0% greater ($P < 0.001$) separable lean than CAS3, and the proportion of total rib-separable lean of CAS8 was 4.7 % greater ($P < 0.001$) than that observed in CAS3. The proportion of total separable lean decreased quadratically ($P < 0.01$) with slaughter age. The proportion of LM in the rib was affected by castration age ($P < 0.001$) and a quadratic effect of slaughter age ($P < 0.001$) was observed. The proportion of LM in the

rib was greater ($P < 0.001$) in bulls compared with castrated animals; and in CAS8 animals this proportion was greater ($P < 0.001$) than CAS3 animals.

Table 3. Ribs ection (9^{th}-11^{th}) and estimated carcass composition of Holstein bulls, bulls castrated at 8 mo of age (CAS8) or at 3 mo of age (CAS3) and slaughtered at 10, 12 and 14 mo of age fed a high-concentrate diet

	Treatment								
	Castration age[1]			Slaughter age[2]				*P-value*[3]	
Item[6]	Bulls	CAS8	CAS3	10	12	14	SEM	CA	SA
Ninth-tenth-eleventh-rib weight[7], kg	4.5	4.4	4.3	3.7^{c}	4.2^{b}	5.3^{a}	0.07	0.16	< 0.001
Ninth-tenth-eleventh-rib cut[8]									
Separable fat[9], %	19.2^{c}	25.7^{b}	28.0^{a}	19.6^{b}	26.3^{a}	27.0^{a}	0.67	< 0.001	< 0.001
Separable subcutaneous fat[5, 10], %	6.4^{b}	8.8^{a}	9.5^{a}	5.7^{c}	10.8^{a}	8.1^{b}	0.44	< 0.001	< 0.001
Separable intermuscular fat[5, 11], %	8.3^{c}	11.1^{b}	12.6^{a}	9.0^{b}	9.4^{b}	13.5^{a}	0.40	< 0.001	< 0.001
Separable internal fat, %	4.5^{b}	5.9^{a}	5.9^{a}	4.9^{b}	6.0^{a}	5.3^{b}	0.21	< 0.001	< 0.001
Separable lean[12], %	58.9^{a}	53.0^{b}	50.8^{c}	56.9^{a}	53.0^{b}	52.8^{b}	0.55	< 0.001	< 0.001
Separable LM [9], %	27.8^{a}	24.5^{b}	22.7^{c}	26.0^{b}	27.8^{a}	21.3^{c}	0.35	< 0.001	< 0.001
Separable remaining lean[9], %	31.1^{a}	28.5^{b}	28.1^{b}	30.9^{a}	25.2^{b}	31.5^{a}	0.42	< 0.001	< 0.001
Separable bone[9], %	21.9^{a}	20.7^{b}	20.6^{b}	22.9^{a}	20.2^{b}	20.1^{b}	0.27	< 0.01	< 0.001
Ninth-tenth-eleventh-rib cut (edible portion)									
Protein[8,12], %	17.3^{a}	15.9^{b}	15.4^{c}	16.8^{a}	15.9^{b}	15.9^{b}	0.13	< 0.001	< 0.001
Ether Extract[8,9], %	22.7^{c}	28.2^{b}	30.2^{a}	23.1^{b}	28.7^{a}	29.3^{a}	0.57	< 0.001	< 0.001

[1] Bulls, CAS3 = bulls castrated at 3 mo of age, CAS8= bulls castrated at 8 mo of age.
[2] 10= animals slaughtered at 10 mo of age, 12= animals slaughtered at 12 mo of age, 14= animals slaughtered at 14 mo of age.
[3] CA= effect of castration age; SA = effect of slaughter age
[6] L $P < 0.01$; Q $P < 0.01$; SA x SA x CA $P = 0.06$
[7] L $P = 0.74$; Q $P = 0.02$
[8] Equations-reference: Hankins and Howe 1946.
[9] L $P < 0.001$; Q $P < 0.001$
[10] L $P < 0.001$; Q $P < 0.001$; SA x SA x CA $P < 0.01$
[11] L $P = 0.01$; Q $P < 0.001$; SA x SA x CA $P = 0.09$
[12] L $P < 0.001$; Q $P < 0.01$

Also, the proportion of remaining lean after LM removal was greater ($P < 0.001$) in bulls compared with castrated animals, and no differences were observed between CAS8

and CAS3 (Table 2). The rib-separable bone proportion was affected by castration age ($P < 0.01$) and quadratically by slaughter age ($P < 0.001$; Table 3), with bulls having a greater ($P < 0.01$) proportion of rib-separable bone compared with CAS8 and CAS3. The proportion of rib-separable bone was greater ($P < 0.001$) in animals slaughtered at 10 mo compared to animals slaughtered at 12 and 14 mo of age. As expected, the carcass content of protein and ether extract estimated by equations described by Hankins and Howe (1946) followed the same pattern of the proportions of separable rib lean and fat, respectively.

3.4. Meat quality

Meat pH was greater ($P < 0.001$) in bulls than in castrated animals independently of castration age (Table 4). Slaughter age had a quadratic effect ($P < 0.001$) on meat pH, with the greatest values being obtained at 12 mo of slaughter age.

The WBSF on day 0 of aging was affected by castration age ($P < 0.01$) and linearly decreased with slaughter age ($P < 0.001$; Table 3). On day 0, WBSF values for bulls were greater ($P < 0.05$) than those for CAS8 and CAS3, but these differences were not observed on day 7 of aging. At day 0 of aging, meat from animals slaughtered at 10 or 12 mo of age was less ($P < 0.001$) tender (greater WBSF values) compared with that from animals slaughtered at 14 mo of age. However, the effect of slaughter age on WBSF was not observed after 7 d of aging.

Meat from CAS3 did not differ in lightness and redness from that of CAS8 (Table 4). However, meat from bulls was darker, less red, and less yellow ($P < 0.001$) compared with the meat from castrated animals. Lightness and redness were non-linearly affected by slaughter age ($P < 0.001$). Meat from animals slaughtered at 10 mo of age was less dark than meat from animals slaughtered at 12 or 14 mo of age; and meat from animals

slaughtered at 12 mo of age was more red than meat from animals slaughtered at 10 or 14 mo of age (Table 4). No significant slaughter age effect was observed for b^* values.

The percentage of LM i.m. fat was affected by a non-linear slaughter age by castration age interaction ($P < 0.001$). In bulls and CAS8, the i.m. fat content increased linearly, whereas in CAS3 it increased quadratically with age. At 10 mo of age, CAS3 (2.1 ± 0.23 %) had a 55.1 % greater ($P < 0.01$) i.m than bulls (1.4 ± 0.23 %) and CAS8 (1.4 ± 0.23 %), and no differences were observed between bulls and CAS8 animals. At 12 mo of age, CAS3 (2.7 ± 0.23 %) had 19.1 % and 65.0 % more i.m. fat than CAS8 (2.3 ± 0.23%) and bulls (1.7 ± 0.23 %), respectively. At 14 mo of age the difference in percentage of i.m. fat between CAS3 (3.9 ± 0.23 %) and CAS8 (3.2 ± 0.23 %) was 18.2% (similar to the differences between CAS3 and CAS8 observed at 12 mo of age); however, the difference between CAS3 and bulls (1.9 ± 0.23 %) increased to 98.5 %. In LM no interaction in protein percentage between slaughter age and castration age were observed. However, animals castrated at 8 mo of age had greater ($P < 0.05$) LM protein percentage than bulls and animals castrated at 3 mo of age (Table 3). Moreover, the proportion of LM humidity was affected by a non-linear slaughter age by castration age interaction ($P < 0.01$). The humidity percentage followed the opposite pattern of i.m fat; in bulls and CAS8 it decreased linearly, whereas in CAS3 it decreased quadratically with age. At 10 mo of age, CAS3 (71.8 ± 0.20 %) and CAS8 (73.9 ± 0.20 %) had less ($P < 0.05$) LM humidity content than bulls (74.5 ± 0.20%) and these differences increased at 12 mo of age. However, at 14 mo of slaughter age, CAS3 animals had less ($P < 0.01$) LM humidity percentage than CAS8 animal, and CAS8 had lesser ($P = 0.05$) LM humidity percentage than bulls (71.8 ± 0.20 % for CAS3, 72.5 ± 0.20 % for CAS8 and 73.8 ± 0.20 % for bulls).

Initial hardness was affected ($P < 0.05$) by a non-linear castration age effect

(Table 5). Initial hardness was greatest at 10 and 12 mo of slaughter age, whereas meat from animals slaughtered at 14 mo had the less initial hardness. Meat from bulls and CAS8 had a greater ($P < 0.001$) initial hardness than that from CAS3. An interaction between castration age and slaughter age tended ($P = 0.06$) to affect overall hardness. In bulls and CAS8 animals, overall hardness decreased linearly with slaughter age, whereas in CAS3 animals slaughter age did not affect overall hardness.

Table 4. Meat quality of LM of Holstein bulls, bulls castrated at 8 mo of age (CAS8) or at 3 mo of age (CAS3) and slaughtered at 10, 12 and 14 mo of age fed a high-concentrate diet

	Treatment								
	Castration age[1]			Slaughter age[2]				*P-value*[3]	
Item[7]	Bulls	CAS8	CAS3	10	12	14	SEM	CA	SA
pH[4]	5.7[a]	5.5[b]	5.5[b]	5.5[c]	5.7[a]	5.6[b]	0.03	< 0.001	< 0.001
WBSF day 0[5, 6], kg	6.6[a]	6.3[ab]	5.7[b]	7.0[a]	6.4[a]	5.2[b]	0.28	0.05	< 0.001
WBSF day 7[5], kg	5.0	5.2	5.3	4.8	5.3	5.3	0.27	0.76	0.41
Instrumental color[7]									
L^*[8]	32.0[b]	33.7[a]	34.0[a]	36.1[a]	24.3[c]	29.3[b]	0.34	< 0.001	< 0.001
a^*[9]	14.5[b]	15.9[a]	15.6[a]	15.2[b]	16.7[a]	14.1[c]	0.26	< 0.001	< 0.001
b^*	1.6[c]	2.3[b]	2.7[a]	2.2	2.4	2.4	0.15	< 0.001	0.46
Intramuscular fat[10, 11], %	1.6[c]	2.3[b]	2.9[a]	1.6[c]	2.2[b]	3.0[a]	0.13	< 0.001	< 0.001
Protein, %	24.3[b]	24.6[a]	24.3[b]	24.5	24.4	24.5	0.09	0.05	0.67
Humidity, [10, 12] %	74.2[c]	73.2[a]	72.8[b]	74.1[a]	73.5[b]	72.7[c]	0.12	< 0.001	< 0.001

[1] Bulls, CAS3 = bulls castrated at 3 mo of age, CAS8= bulls castrated at 8 mo of age.
[2] 10= animals slaughtered at 10 mo of age, 12= animals slaughtered at 12 mo of age, 14= animals slaughtered at 14 mo of age.
[3] CA= effect of castration age; SA = effect of slaughter age
[4] L $P < 0.01$; Q $P < 0.001$
[5] Warner–Bratzler shear force
[6] L $P < 0.001$; Q $P = 0.42$
[7] Color: L* = lightness, a* = redness, and b* = yellowness.
[8] L $P = 0.08$; Q $P < 0.001$
[9] L $P < 0.001$; Q $P < 0.001$
[10] Interaction between castration age and slaughter age (intramuscular fat $P = 0.06$; humidity $P = 0.04$)
[11] L $P = 0.60$; Q $P = 0.58$; SA x SA x CA $P < 0.05$
[12] L $P = 0.62$; Q $P = 0.51$; SA x SA x CA $P < 0.01$

At 10 and 12 mo of slaughter age meat from bulls (6.1 and 6.1 ± 0.09, respectively) and CAS8 (6.1 and 6.1 ± 0.09) had greater ($P < 0.05$) hardness values than that from CAS3 (5.8 and 5.6 ± 0.09, respectively). At 14 mo of age, meat from castrated animals, independently of castration age, was less ($P < 0.05$) hard (5.6 and 5.5 ± 0.09, for CAS8 and CAS3, respectively) than meat from bulls (5.8 ± 0.09). Juiciness was affected by a quadratic interaction between slaughter age and castration age ($P < 0.05$). Meat from bulls and CAS3 had 6.7 and 5.0 % greater ($P < 0.05$) juiciness than that from CAS8. No differences in meat juiciness were observed between 10 and 12 mo of slaughter age; however, meat from animals slaughtered at 14 mo of age had greater ($P < 0.01$) meat juiciness than meat from animals slaughtered at younger ages (Table 5). Meat from CAS3 tended ($P = 0.09$) to have less flavor compared to meat from bulls and CAS8.

Table 4. Sensory quality of the LM from Holstein bulls, bulls castrated at 8 mo of age (CAS8) or at 3mo of age (CAS3) and slaughtered at 10, 12 and 14 mo of age fed a high-concentrate diet

	Treatment								
	Castration age[1]			Slaughter age[2]				*P-value*[3]	
Item	Bulls	CAS8	CAS3	10	12	14	SEM	CA	SA
Initial hardness [4]	5.9[a]	5.8[a]	5.5[b]	5.8[a]	5.8[a]	5.5[b]	0.17	< 0.001	< 0.001
Overall hardness [5,6]	6.0[a]	5.9[a]	5.6[b]	6.0[a]	5.9[a]	5.6[b]	0.17	< 0.001	< 0.001
Juiciness [7]	2.7[a]	2.5[b]	2.6[a]	2.5[b]	2.6[b]	2.7[a]	0.30	0.01	0.03
Flavor	2.6	2.5	2.4	2.4	2.5	2.6	0.10	0.09	0.34

[1] Bulls, CAS3 = bulls castrated at 3 mo of age, CAS8= bulls castrated at 8 mo of age.
[2] 10= animals slaughtered at 10 mo of age, 12= animals slaughtered at 12 mo of age, 14= animals slaughtered at 14 mo of age.
[3] CA= effect of castration age; SA = effect of slaughter age
[4] L $P = 0.11$; Q $P = 0.03$
[5] Interaction between castration age and slaughter age (overall hardness $P = 0.06$)
[6] L $P = 0.19$; Q $P = 0.05$
[7] L $P < 0.22$; Q $P = 0.05$

4. DISCUSSION

4.1. Effect of castration age

In the present study, castration decreased ADG and feed efficiency. Different authors (Martin and Stob, 1978, Fisher et al., 2001; Earley and Crowe, 2002) have reported that bulls gain more rapidly and efficiently than steers. The greater ADG observed in bulls compared with steers could be attributed to the anabolic property of androgens, especially testosterone (Galbraith et al., 1978; Katz, 2007; Mach et al., 2009). Previous studies (Brännäng, 1966; Hedrick, 1969; Field, 1971) indicated that bulls grow on average 14 to 17% more than steers; however, in the present study castration only reduced ADG a 7.5**%** compared with bulls.

It was expected that bulls castrated after puberty would have a greater ADG and final BW than bulls castrated at 3 mo of age, because when castration is performed after puberty the advantages of bulls on performance could be maintained for a longer period of time throughout the growing phase (Knight et al., 1999a). However, surgical castration has an important detrimental effect on performance during 2 wk after castration (Devant et al., 2012) and this slump probably offsets the expected advantages of delaying age of castration on performance. In agreement to the present study, different studies conducted with different breeds (precocious and late-maturing) and feeding systems compared with the present study did not report an effect of castration age on performance (Klosterman et al., 1954; Champange et al., 1969; Worrell et al., 1987; Mellor et al., 1991; Parrasin et al., 1999; Micol et al., 2009). As castration did not affect concentrate intake or nutrient intake expressed as percentage of metabolic BW, detrimental effects of castration on feed efficiency were mainly due to its negative effect on performance. In accordance with the present study, Mach et al. (2009) and Marti et al. (2011) did not observe differences in feed consumption between bulls and animals castrated at 7 and 3 mo of age. In addition,

Champange et al. (1969) and Klosterman et al. (1954) did not report differences in concentrate intake among animals castrated at different ages. Detrimental effects of castration on feed intake are usually described for the few weeks after surgery (Fisher et al., 1996; Ting et al., 2003; Devant et al., 2012), but these are transitory and have a small impact on overall feed intake, as also observed in the present study. The quantification of the detrimental effects of castration on performance and feed efficiency is crucial to estimate production costs and to decide if castration is an economically sound strategy.

The WBSF values at d 0 and d 7 observed in the present study are close to the observed in Holstein bulls and steers fed high-concentrate diets (Mach et al., 2009; Marti et al., 2011). As expected, castration improved meat tenderness and meat pH (lower values). Also meat of castrated animals had more lightness, redness and yellowness than meat from bulls. In addition, bulls may be stressed more easily (Field, 1971; Katz, 2007) and perform more mounting activity (Katz, 2007; Mach et al., 2009) than steers. These two factors may explain the greater meat pH and the darkness observed in bulls compared with the meat from steers (Jago et al., 1996; Price et al., 2003; Katz, 2007). In accordance to the results presented herein, several authors observed that meat of castrated animals at pre-pubertal ages (Purchas et al., 2002; Marti et al., 2011) or at post-pubertal ages (Mach et al., 2009) has lower WBSF values and consequently lesser hardness compared with meat from bulls. These authors associated the increase of tenderness with a slightly lower ultimate pH, greater myofibrilar fragmentation index, greater i.m. fat content, and possibly, a smaller contribution of connective tissue. However, after 7 d of aging, differences in WBSF values disappeared as also reported by Cahill et al. (1964). The small juiciness differences observed between castrated animals and bulls in the present study have also been previously reported (Purchas et al., 2002; Mach et al., 2009; Marti et al., 2011).

Castration, independently of slaughter age, increased rib separable fat content and decreased rib separable lean content. In addition, in the present study, it was also observed that castration age affected carcass-separable fat and lean, and estimated carcass protein and ether extract content. The younger the animals were castrated, independently of slaughter age, the greater the rib-separable fat percentage (carcass ether extract) and the lesser rib-separable lean percentage (carcass protein) were deposited. Also, the amount and location of fat is important because internal, intermuscular, and s.c. fats are of low economic value, and only i.m. fat is appreciated by consumers (Aldai et al., 2007). Intermuscular separable fat was greater in castrated animals compared with bulls as described by Keane et al. (2003), and when Holstein bulls were castrated at 3 mo of age this intermuscular percentage was greater than when castration was delayed at 8 mo of age**.** In addition, internal separable fat increased in castrated animals compared with bulls independently of castration age. Internal fat during carcass manipulation usually is removed, and in consequence carcass dressing percentage may be impaired, as observed with castrated animals in the present study.

4.2. Effect of slaughter age

As described by May et al. (1992), final BW and carcass weight improved as slaughter age increased. Berg and Butterfield (1968) observed, in both beef and milk type breeds, that weight at slaughter has an important influence on carcass composition, the point of inflexion of carcass fat deposition is approximately between 12 and 18 mo of age. In the present study, rib section data evolution, which reflects carcass composition evolution (Hankins and Howe, 1946), was in accordance to several studies (Berg and Butterfield, 1968; Jenkins et al., 1981; Andersen and Ingvartsen, 1984). As rib weight increased with slaughter age, muscle and bone percentages decreased and fat percentage

increased. However, in the present study, this evolution was only observed from 10 to 12 mo of age, and no differences between 12 and 14 mo of slaughter age were observed, so in Holstein animals fed high-concentrate diets the inflexion of carcass fat deposition would be around 10 and 12 mo of age. Differences among studies could be related to breed and/or feeding systems.

On the other hand, as reported by Van Koevering et al. (1995) concentrate intake increased linearly with slaughter age. Decreasing slaughter age, independently of castration, may be a good strategy to reduce production costs when feed prices are high; however, the quantification of the detrimental effects of reducing slaughter age on carcass weight is necessary to decide whether this strategy has negative effects on net returns. In addition, performing the slaughter at 10 mo of age may improve meat pH. Meat with pH above 6.0 at 24 h after slaughter represents a meat quality problem and is undesirable for consumption (Pipek et al., 2003; Viljoen et al., 2002; Wulf et al., 2002). Mellor et al. (1991) observed that temperament and stress level increased with age, and as a consequence animals slaughtered at older ages have increased meat pH and lower L^* values.

As described by Hedrick et al. (1969), it was expected that age would have an adverse effect on tenderness. The negative effect of age on tenderness is mainly attributed to an increase in intermuscular collagen, which becomes progressively tougher, more rigid and resistant, and less easily denatured as age increases (Nishimura et al., 1999). In the present study tenderness improves by age and one possible explanation for these unexpected results, could be that Hedrick et al. (1969) used animals that grazed for a long period and/or because animals were slaughtered at older ages (between 15 and 18 mo) while in contrast in the present study these Holstein bulls and steers were fed concentrate and straw throughout the study and were slaughtered at the maximum of 14 mo of age.

4.3. Castration age and slaughter age interactions

In the present study, most of the interactions between castration age and slaughter age were observed in carcass classification, carcass dressing percentage, and in some rib fat distribution characteristics. Nutrient intake can affect the effect of castration age on carcass composition (Mellor et al., 1991); however in the present study nutrient intake, daily consumption or expressed as percentage of metabolic BW, was not affected by the castration age, discarding the possible confounding effect between nutrient intake and castration on carcass composition. When castration was performed in the pre-pubertal period, carcass conformation was poor independently of slaughter age, probably because anabolic effects of testosterone were suppressed at an early stage when most of muscle growth takes place. Marti et al. (2011) also found greater conformation grades in bulls compared with pre-pubertal castrated animals. However, Andersen and Ingvartsen (1984) did not find differences between animals castrated at 3-4 mo of age and bulls. As in the present study, Mach et al. (2009) did not find a castration effect on carcass conformation when castration was performed at post-puberty ages and animals were slaughtered at 12 mo of age. The improvement of carcass conformation in CAS8 Holstein animals between 10 and 12 mo of slaughter age was greater than in the other treatments because at 10 mo of age these animals did not recover completely from surgical castration. Delaying slaughter age is a good strategy to improve carcass conformation when Holstein animals are castrated at 8 mo of age, but does not seem to be an effective strategy when Holstein animals are castrated at 3 mo of age.

When carcass fat cover is the main carcass quality trait to be improved, as it is the case in some European markets, castration at young ages is a good strategy to slaughter Holstein animals at young ages because the desired carcass fat cover of “3” could be achieved at 10 mo of age. However, as mentioned before, if Holstein animals are

castrated at pre-pubertal ages and slaughtered at young ages, carcass conformation will be impaired. These statements are valid for Holstein bulls fed high-concentrate diets slaughtered around one year of age; however, breeds like Charolais cattle need longer to deposit enough adipose tissue and reach their optimal slaughter stage than cattle from a precocious breed. These characteristics might appear to be in conflict with the shortening of the production cycle. However, Micol et al. (2009) studied if the castration of Charolais bulls at a young age (2 mo of age) rather than at 10 months could enable the fattening of the steers to be speeded up and also allow slaughtering at a younger age (28 mo of age versus 36 mo of age). These authors (Micol et al., 2009) did not observe any detrimental effect in Charolais bulls of reducing castration age from 10 to 2 mo of age on performance and carcass characteristics when animals were slaughtered at 26 to 28 mo of age. Champagne et al. (1969) in yearling Herefords observed that the animals castrated at 9 mo of age and slaughtered 9 mo later had a decreased carcass conformation score compared to those castrated at birth, or at 2 mo of age. So, breed, feeding system, and interval between castration and animal marketing are crucial factors to evaluate the optimum age of castration on performance and carcass characteristics (Mellor et al., 1991).

Dressing percentage improved as days on feed increased, as it has also been previously reported (Schroeder et al., 1980, Tatum et al., 1980; May et al., 1992). Dressing percentage of CAS8 animals slaughtered at 10 mo of was low because they probably did not fully recover from castration. In CAS3 animals, dressing percentage was less than that of bulls at 10 mo of age because their carcasses were fatter (as indicated by the greater level of carcass fat cover and rib dissection data) and had probably more fat removal during carcass manipulation (excess of fat in the kidney, heart, and pelvis) than carcasses of bulls. Field et al. (1971) summarized different studies that evaluated the

effect of castration on performance and carcass quality, and they observed no clear effect of castration on dressing percentage. However, in most studies (Field et al., 1971) where fat depth was increased due to castration, dressing percentage was greater in bulls than steers, supporting the hypothesis that carcasses with great fat cover could have more fat retails during carcass manipulation. Moreover in accordance to the present study, other authors (Champagne et al., 1969; Adams and Adams, 1992; Huxsoll et al., 1998) have also reported lower dressing percentages of steers compared with bulls.

Several authors (Berg and Butterfield, 1968; Jenkins et al., 1981; Andersen and Ingvartsen, 1984) have indicated the percentage s.c. fat increases with slaughter age. However, in the present study, at 14 mo of age the proportion of rib-separable s.c. fat decreased and the proportion of rib-separable intermuscular fat increased, and this effect was most pronounced in CAS3 Holstein animals. In pre-pubertal castrated Holstein animals, the age of slaughter should be reduced to 10 mo of age because older ages produced an undesirable increase in the percentage of intermuscular fat depots, and consequently meat from these animals could be refused by consumers. As mentioned before, reducing slaughter age to 10 mo in pre-pubertal castrated Holstein animals has advantages such as improving carcass fat cover and reducing total feed cost, and disadvantages because carcass conformation is impaired.

The increase of i.m. fat is desired as it may improve meat tenderness by reducing bulk density and decreasing the effect of the strength of the connective tissue (Savell and Cross, 1988), also large amounts of i.m fat can increase meat lightness (Boucqué et al., 1982; Fiems et al., 2000) as observed in castrated animals. However, the i.m fat in CAS8 animals did not increase until 12 mo of age, probably as a consequence of the stress produced by surgical castration (Knight et al., 1999b; Bretschneider, 2005; Devant et al., 2012). In addition, bulls should be slaughtered at 14 mo of age to achieve similar i.m. fat

levels compared to CAS3 animals slaughtered at 10 mo of age. The percentage of i.m. fat was greater when animals were castrated at young ages at all slaughter ages as also reported by Champagne et al. (1969) and Andersen and Ingvartsen (1984). Therefore, when marbling is an important meat quality attribute, as in the North-American market, Holstein animals castrated before puberty could be slaughtered at younger ages (less than one year old) compared to post-puberty castrated animals and bulls without impairing meat marbling, and consequently feed and production costs could be potentially reduced. This method of castrating steers at a young age is commonly used in Anglo- Saxon countries, with breeds such as Angus and Hereford. In agreement with the present study, Worrell et al. (1987) observed in Angus×Hereford steers slaughtered at 470 kg that castration at of 70 or 230 kg of BW improved marbling score compared with castration at 320 or 410 kg of BW. However, Destefanis et al. (2003) observed in Piemontese animals slaughtered at 19 mo of age an improvement in i.m. fat when they were castrated at 13 mo of age compared with animals castrated at 5 mo of age. So, the positive effect of castrating animals at young ages to improve marbling seems a good strategy in precocious breeds.

In the present study, early-castrated animals had a reduced overall hardness independently of slaughter age compared with bulls or animals castrated around puberty. In agreement with the present results, Worrell et al. (1987) observed in Angus×Hereford steers slaughtered at 15 mo of age and 470 kg of BW that, meat of the early-castrated steers (70 kg of BW) was more tender than meat of those castrated later (230, 320 and 410 kg of BW). However, Destefanis et al. (2003) observed no positive effect of castration age in Piemontese animals or Micol et al. (2009) with Charolais animals slaughtered at 19 mo of age or 26 mo of age, respectively, on meat tenderness or sensory

attributes. So, early-castration of precocious breeds and slaughtering them young could have an interesting meat tenderness improvement reducing production costs.

In summary, Holstein animals castrated at pre-puberty ages could be slaughtered at younger ages as i.m. fat and carcass fat cover improved. In consequence, the days on feed and the production costs could be reduced. However, pre-puberty castration impairs carcass conformation of Holstein animals if slaughter age is not delayed until 14 mo of age. Surgical castration of Holstein animals performed at 8 mo of age is not a good strategy when combined with reduced slaughter ages, as carcass and meat quality at 10 mo of age are impaired because animals have not fully recovered from castration sequels. In Holstein bulls, to improve meat and carcass quality, slaughter age cannot be reduced to 10 mo of age, as carcass fat cover, i.m. fat and proportion of intermuscular fat in the rib are excessively low. Moreover, Holstein bulls have to be slaughtered at 14 mo of age if the same i.m. fat of the LM than animals castrated at 3 mo of age and slaughtered at 10 mo of age is to be achieved.

5. IMPLICATIONS

Castration of Holstein bulls, regardless of castration age and slaughter age, impairs animal growth and feed efficiency, reduces meat pH, WBSF at day 0 of aging, and increases rib-separable i.m fat. Meat from animals castrated at 3 mo of age has less overall and initial hardness than meat of animals castrated at 8 mo of age and bulls. Moreover, when Holstein animals are castrated before puberty, independently of slaughter age, rib-separable fat percentage increases and rib-separable lean percentage decreases. In Holstein animals, slaughter age, independently of castration, enhances feed consumption and impairs feed efficiency. However, in Holstein animals slaughter age

depending on castration age affects differently parameters related to fat deposition such as dressing percentage carcass fat cover, and rib fat distribution.

6. LITERATURE CITED

Adams, T. E., and M. Adams. 1992. Feedlot performance of steers and bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 70:1691-1698.

Aldai, N., A. I . Nájera, M. E. R. Dugan, R. Celaya, and K. Osoro. 2007. Characterization of intramuscular, intermuscular and subcutaneous adipose tissue in yearling bulls of different genetic groups. Meat Sci. 76:682-691.

Amer, P. R., R. A. Kemp J. G. Buchanan-Smith, G. C. Fox, and C. Smith. 1994. A bioeconomic model for comparing beef cattle genotypes at their optimal economic slaughter end point. J. Anim. Sci. 72:38-50.

Andersen, H. R., and K .L. Ingvartsen. 1984. Influence of energy level, weight at slaughter and castration on carcass quality in cattle. Livest. Prod. Sci. 11:571-586.

AOAC. 1995. Oficial Methods of Analysis. 16th ed. Association of Official Analytical Chemist, Arlington, VA.Arthaud, V. H., R. W. Mandigo, R. M. Koch and A. W. Kotula. 1977. Carcass composition, quality and palatability attributes of bulls and steers fed different energy levels and killed at four ages. J. Anim. Sci. 44:53-64.

Brännäng, E. 1966. Studies on mozygous cattle twins XVIII. The effect of castration and age of castration on the growth rate, feed conversion and carcass traits of Swedish red and white cattle. Part I. Lantbrukshögsk Ann. 32:329-415.

Berg, R. T. and R. M. Butterfield. 1968. Growth patterns of bovine muscle, fat and bone. J. Anim. Sci. 27:611-619.

Boucqué Ch. V., L. O. Fiems, B. G. Cottyn, M. Casteels, and F. X. Buysse. 1982. Monensin-sodium as a performance-promoting additive for fattening bulls and its impact on carcass and meat quality characteristics. Anim. Feed Sci. Technol. 7:401-410.

Bretschneider, G. 2005. Effects of age and method of castration on performance and stress response of beef male cattle. A review. Livest. Prod. Sci. 97:89-100.

Cahill, V. R. 1964. Effects of sex differences in beef carcasses relative to cutability and palatability. Proc. Recip. Meat. Cont. 17:35.

Champagne, J. R., J. W. Carpenter, J. F. Hentges, Jr., A. Z. Palmer, and M. Koger. 1969. Feedlot performance and carcass characteristics of young bulls and steers castrated at four ages. J. Anim. Sci. 29:887-890.

Commission Internationale de l'E´ clairage (1976). Colorimetry. Publication nº_15, Bureau Central de la CIE, Vienna, Austria.Field, R. A., 1971. Effect of castration on meat quality and quantity. J. Anim. Sci. 32:849-858.

Destefanis, G., A. Brugiapaglia, M. T. Barge, and C. Lazzaroni. 2003. Effect of castration on meat quality in Piemontese cattle. Meat Sci. 64:215-218.

Devant, M., S. Marti, and A. Bach. 2012. Eating pattern of Holstein bulls and steers fed high-concentrate rations using a computerized concentrate feeder. J. Anim. Sci. Online June. http://jas.fass.org/content/early/2012/06/13/jas.2011-4929.

Earley, B., and M. A. Crowe. 2002. Effects of ketoprofen alone or in combination with local anesthesia during castration of bull calves on plasma cortisol, immunological, and inflammatory responses. J. Anim. Sci. 80:1044-1052.

Field, R. A., 1971. Effect of castration on meat quality and quantity. J. Anim. Sci. 32:849-858.

Fiems, L. O., S. De Campeneere, S. De Smet, G. Van de Voorde, J. M. Vanacker, and Ch.V. Bounqué. 2000. Relationship between fat depots in carcasses of beef bulls and effect on meat colour and tenderness. Meat Sci. 56:41-47.

Fisher, A. D., M. A. Crowe, M. E. Alonso de la Varga, and W. J. Enright. 1996. Effect of castration method and the provision of local anesthesia on plasma cortisol, scrotal circumference, growth, and feed intake of bull calves. J. Anim. Sci. 74:2336–2343.

Fisher, A. D., T. W. Knight, G. P. Gosgrove, A. F. Death, C. B. Anderson, D. M. Duganzich, and L. R. Matthews. 2001. Effect of surgical or banding castration on stress responses and behavior of bulls. Asut. Vet. J. 79:279-284.

Galbraith, H., D. G. Demspter, and T. B. Miller. 1978. A note on the effect of castration on the growth performance and concentration of some blood metabolites and hormones in British Friesian male cattle. Anim. Prod. 26:339-342.

Hankins, O. G., and P. E. Howe. 1946. Estimation of the composition of beef carcasses and cuts. Tech. Bull. 926:1-19.

Hedrick, H. B., G. B. Thompson and F. Krause. 1969. Comparison of feedlot performance and carcass characteristics of half-sib bulls, steers and heifers. J. Anim. Sci. 29:687-694.

Huxsoll, C. C., E. O. Price, and T. E. Adams. 1998. Testis function, carcass traits, and aggressive behavior of beef bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 76: 1760-1766.

Jago, J. G., L. R. Matthews, J. J. Bass, and T. W. Knight. 1996. A comparison of two methods of castration of post-pubertal beef cattle and their effect on behavior, growth and ultimate pH. Proc. N.Z. Soc. Anim. Prod. 56:395-397.

Jenkins, T. G., C. R. Long, T. C. Cartwright, and G. C. Smith. 1981. Characterization of cattle of a five-breed diallel. IV. Slaughter and carcass characteristics of serially slaughtered bulls. J. Anim. Sci. 53:62-79.

Katz, L. S. 2007. Sexual behavior of domesticated ruminants. Horm. Behav. 52:56-63.

Keane, M. G. 2003. Beef production from Holstein-Friesian bulls and steers of New Zealand and European/American descent, and Belgian Blue x Holstein-Friesian, slaughtered at two weights. Livest. Prod. Sci. 84:207-218.

Klosterman, E. W., L. E. Kunkle, P. Gerlaugh, and V. R. Cahill. 1954. The effect of age of castration upon rate and economy of gain and carcass quality of beef calves. J. Anim. Sci. 13:817-825.

Knight, T. W., G. P. Cosgrove, A. F. Death, and C. B. Anderson. 1999a. Effect of method and age at castration on growth rate and meat quality of bulls. N.Z.J. Agric. Res. 42:255-268.

Knight, T. W., G. P. Cosgrove, A. F. Death, and C. B. Anderson. 1999b. Effect of interval from castration of bulls to slaughter on carcass characteristics and meat quality. N.Z.J. Agric. Res. 42:269-277.

Mach, N., A. Bach, C. E. Realini, M. Font i Furnols, A. Velarde, and M. Devant. 2009. Burdizzo pre-pubertal castration effects on performance, behavior, carcass characteristics, and meat quality of Holstein bulls fed high-concentrate diets. Meat Sci. 81:329-334.

Mark, D. R., T. C. Schroeder, and R. Jones. 2000. Identifying economic risk in cattle feeding. J. Agribus. 18:331-344.

Marti, S., A. Velarde, J. L. de la Torre, A. Bach, A. Aris, A. Serrano, X. Manteca, and M. Devant. 2010. Effects of ring castration with local anesthesia and analgesia in Holstein calves at 3 month of age on welfare indicators. J. Anim. Sci. 88:2789-2796.

Marti, S., C. E. Realini, A. Bach, M. Pérez-Juan, and M. Devant. 2011. Effect of vitamin A restriction on performance and meat quality in finishing Holstein bulls and steers. Meat Sci. 89:412-418.

Martin, T. G., and M. Stob. 1978. Growth and carcass traits of Holstein steers, bulls and bulls implanted with Diethylstilbestrol. J. Dairy. Sci. 61:132-134.

May, S. G., H. G. Dolezal, D. R. Gill, F. K. Ray, and D. S. Buchanan. 1992. Effects of days fed, carcass grade traits, and subcutaneous fat removal on postmortem muscle characteristics and beef palatability. J. Anim. Sci. 70:444-453.

McFie, H. J., and D. M. Thompson. 1988. Preference mapping and multidimensional scaling. In J. R. Piggot (Ed.), Sensory analysis of foods. London: Elsevier Applied Science.

Mellor, D. J., V. Molony, I. S. Robertson. 1991. Effects of castration on behavior and plasma cortisol concentrations in young lambs, kids and calves. Res. Vet. Sci. 51:149-154.

Micol , D., M. P. Oury, B. Picard, J. F. Hocquette, M. Briand, R. Dumont, D. Egal, R. Jailler, H. Dubroeucq, and J. Agabriel. 2009. Effect of age at castration on animal performance, muscle characteristics and meat quality traits in 26-month-old Charolais steers. Livestock Sci. 120 :116-126.

Nishimura, T., A. Hattori, and K. Takahashi. 1999. Structural changes in intramuscular connective tissue during the fattening of Japanese black cattle: effect of marbling on beef tenderization. J. Anim. Sci. 77:93-104.

Parrisin, P. R., V. Thénard, R. Dumont, M. Grosse, J. M. Trommenschlager, and M. Roux. 1999. Effet d'une castration tardive sur la production de bœuf Holstein et Montbéliards. INRA Prod. Anim. 12:207-216.

Pipek, P., A. Haberl, and J. Jeleniková. 2003. Influence of slaughterhouse handling on the quality of beef carcasses. Czech J. Anim. Sci. 9:371-378.

Price, E. O., T. E. Adams, C. C. Huxsoll, and R. E. Borqwardt. 2003. Aggressive behavior is reduced in bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 81:411-415.

Purchas, R. W., D. L. Burnham, and S. T. Morris. 2002. Effect of growth potential and growth path on tenderness of beef *longissimus* muscle from bulls and steers. J. Anim. Sci. 80:3211-3221.

Pyatt, N. A., L. L. Berger, D. B. Faulkner, P. M. Walker, and S. L. Rodriguez-Zas. 2005. Factors affecting carcass values and profitability in early-weaned Simmental steers: II. Days on feed endpoints and sorting strategies. J. Anim. Sci. 83:2926-2937.

Savell, J. W., and H. R. Cross. 1988. The role of fat in the palatability of beef, pork and lamb. In Dressing foods. Animal product options in the market place. National Academy Press, Washington D.C. 345-355.

Schroeder, J. W., D. A. Cramer, R. A. Bowling, and C. W. Cook. 1980. Palatability, shelflife and chemical differences between forage- and gain- finished beef. J. Anim. Sci. 50: 852-859.

Serra, X., L. Guerrero, M. Guàrdia, M. Gil, C.Sañudo, B. Panea, M. Campo, J. Olleta, M. García-Cachán, J. Piedrafita, and M. Oliver. 2008. Eating quality of young bulls from three Spanish beef breed-production systems and its relationship with chemical and intrumental meat quality. Meat Sci. 79: 98-104.

Tatum, J. D., G. C. Smith, B. W. Berry, C. E. Murphe, F. L. Williams and Z. L. Carpenter. 1980. Carcass characteristics, time on fed and cooked beef palatability attributes. J. Anim. Sci. 50:833-840.

Ting, S. T. L., B. Earley and M. A. Crowe. 2003. Effect of repeated ketoprofen administration during surgical castration of bulls on cortisol, immunological function, feed intake, growth, and behavior. J. Anim. Sci. 81:1253-1264.

Van Koevering, M. T., D. R. Gill, F. N. Owens, H. G. Dolezal, and C. A. Strasia. 1995. Effect of time on feed on performance of feedlot steers, carcass characteristics, and tenderness and composition of *longissimus* muscle. J. Anim. Sci. 31:307-309.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3588-3597.

Viljoen, H. F., H. L. De Kock and E. C. Webb. 2002. Consumer acceptability of dark firm and dry (DFD) and normal pH beef steaks. Meat. Sci. 61:181-185.

Walstra, P., and P. Merkus. 1995. Procedure for assessment of the lean meat percentage as a consequence of the new EU reference dissection method in pig carcass classification. ID-DLO. Zeist. The Netherlands.

Worrell, M. A., D. C. Clanton, and C. R. Calkins. 1987. Effect of weight at castration on steer performance on the feedlot. J. Anim. Sci. 64:343-347.

Wulf, D. M., R. S. Emnet, J. M. Leheska, and S. J. Moeller. 2002. Relationship among glycolytic potential, dark cutting (dark, firm, and dry) beef, and cooked beef palatability. J. Anim. Sci.80:1895-1903.

Chapter V

EFFECT OF GONADOTROPIN-RELEASING HORMONE VACCINE BOPRIVA® AND BAND CASTRATION ON BEEF CATTLE ON WELFARE INDICATORS

ABSTRACT

Angus bulls (n = 60; 257 d of age; initial BW 358.8 ± 3.98 kg) were used to study the effect of an anti-GnRH vaccine and band castration on indicators of animal welfare. Cattle were randomly assigned to 1 of 3 treatments: Bulls, band-castrated animals without pain mitigation (Castrated), and animals administered an anti-GnRH vaccine Bopriva® (Vaccinated). Animals were randomly assigned to one of 6 pens and were fitted with a radio frequency ear tag so that individual animal feed intake could be daily recorded using an electronic feed bunk monitoring system. Two doses of Bopriva® were administrated on d -35 and 0, and band castration was performed on d 0. Every 7 d until d 56 of the study the BW was recorded, and blood samples were collected for serum testosterone concentration and GnRH IgG antibody titers analysis. Visual analog score (VAS), indicative of pain or discomfort, was used to visually assess the behavioral responses of the bulls to the treatments on d -36, -35, -1, and 0 and salivary cortisol concentration was determined on d -35 and 0 as well as at -30, 0, 30, 60, 120, and 270 min post castration. Hair samples were collected every 28 d analyze hair cortisol concentration. Blood samples were collected on d 1, 2, 5, 7 and weekly, for determination of complete blood count (CBC) and behavior was recorded from d 0 to d 7. Data were analyzed using a mixed-effects model with castration, time and their interactions as main effects. No treatment differences in salivary cortisol or VAS (P = 0.76 and P = 0.33, respectively) were observed on d -35. However, on d 0, band-castrated calves had greater ($P < 0.05$) salivary cortisol concentrations (4.6 ± 0.45 nmol/L) than bulls or vaccianted calves (3.1 ± 0.45 and 3.3 ± 0.45 nmol/L, respectively). Also, VAS assessed on d 0, was 78.2 and 78.9 % greater ($P < 0.001$) in band-castrated than bulls and vaccinated calves, respectively. Postures related to pain as foot stamping or tail wagging were greater ($P < 0.05$) in band-castrated animals than bulls or vaccinated animals. No treatment

differences in hair cortisol concentration were observed among treatments. Therefore, band castration resulted in greater salivary cortisol and VAS scores at day of castration and some behavior traits the days following castration compared to vaccinated animals and bulls, indicating that this procedure caused acute pain or discomfort. Administration of Bopriva® may be a welfare friendly alternative to traditional castration methods in beef cattle.

Key words: Beef, anti-GnRH vaccine, Welfare

1. INTRODUCTION

For centuries, meat producing animals have been castrated because of management difficulties produced by the sexual and aggressive behavior of bulls. However, in recent years public concern regarding the pain associated with traditional castration methods has increased. In addition, the aggressive behavior and libido of male cattle has been shown to increase carcass bruising and dark-cutting meat (Mach et al., 2009). There is some evidence that the pain associated with castration is reduced if the procedure is done at an early age (Bretschneider, 2005). Knight et al. (1999) proposed post-pubertal castration as a management strategy to facilitate optimal growth rates in bulls until the time of castration followed by a period as steer to enhance meat quality. Several different techniques are used to castrate post-pubertal bulls. Burdizzo castration is not always effective (Mach et al. 2009) and surgical castration is associated with acute pain (Molony et al. 1995), infection and bleeding (Turner and McIlwraith, 1989) and in some cases with the death of the animal (Gregory and Ford, 1983; Vanderwert et al., 1985). Consequently, band castration has become the most common castration method used for post-pubertal bulls. However, some studies indicate that this method can produce chronic pain (Molony et al., 1995; Thüer et al., 2007) and may increase financial cost as an anti-tetanus vaccination and antibiotics are recommended to accompany the band castration procedure (Pang et al., 2008). For these reasons an anti-GnRH (Bopriva®) vaccine has been proposed as an animal welfare friendly alternative to reduce sexual and aggressive behavior of intact bulls and eliminates the need for physical castration. Animals vaccined with Bopriva® produce antibodies against-GnRH resulting in reduced plasma testosterone concentrations. To date, there are no published studies evaluating an anti-GnRH vaccine on behavioral or physiological indicators of calf welfare. The objective of

this study was to compare the effects of an anti-GnRH vaccine and band castration on the welfare of Angus crossbreed bulls.

2. MATERIALS AND METHODS

All procedures described within this study were approved by the Animal Care and Use Committee of the Lethbridge Research Centre and according to the guidelines established by the Canadian Council on Animal Care (1993).

2.1. Animals, Housing, and Diets

Sixty Angus and Angus crossbred calves (257 d of age; initial BW 358.8 ± 3.98 kg) came from the same herd at the Agriculture and Agri-Food Canada One-Four experimental ranch located in the southeastern corner of Alberta, Canada. Calves were blocked by BW and age and assigned to 3 treatments, bulls, band-castrated (Callicrate Bander, No-Bull Enterprises Inc., St. Francis, KS) animals without pain mitigation (castrated), and animals vaccinated (Bopriva®, Pfizer Animal Health, Parkville, Australia) with an anti-GnRH vaccine (vaccinated). Calves were randomly assigned to 1 of 6 pens (2 pens/treatment) and were left to adapt to their pens and feed for 1 mo prior to study commencement. Each outdoor pen measured 21 × 27 m and was protected with windbreak fencing, contained a centrally located water system (Bolhmann Inc., Denison, IA) and had a concrete apron (2.4 x 24.5 m) directly in front of the feeders. Straw bedding was added as needed in one corner of all pens. All animals were identified with radio frequency ear tags (Allflex Canada, St-Hyacinthe, Canada). Two electronic feeders per pen allowed automatic recording of individual feed intake and time at the feeder (GrowSafe Systems Ltd, Airdrie, Alberta, Canada). Each feeder measured 0.91 x 0.38 x 0.53 m (height x depth x width), was mounted onto 2 load cells, and allowed only 1 calf to eat at a time. A reader panel recorded readings every second and identified the

transponder number present above the feeder, feeder number, feed weight, and time of day. Cattle were fed ad libitum a total mixed back-grounding ration (57.6 % DM) intake consisting of 61.5% barley silage, 16.4% rolled barley, 17.1% rolled oats, and 5% supplement containing minerals and vitamins (DM basis). Feed was delivered twice daily, and fresh water was available at all times.

Vaccinated calves were administered 1 mL of Bopriva® on d -35 and d 0. Each dose contained 400 µg of a conjugated modified GnRH peptide covalently linked to a carrier protein, together with Advasure, an aqueous adjuvant. Bulls and castrated calves were administered of 1 mL of a 0.9% saline solution (Pfizer Animal Health, Parkville, Australia) on d -35 and also in bulls on d 0 also to serve as a sham injection. Band castration was performed on d 0 of the study. Both the vaccine and saline solutions were administered subcutaneously on the lateral aspect of the left side of the neck using a ½ inch 16 gauge needle using a safety vaccinator (Simcro Safety Auto, Simcro, New Zealand) to prevent inadvertent self-administration; this safety vaccinator tented the skin of the animals, facilitating administration with one hand and ensuring consistent delivery.

2.2. Measurements and Sample Collection

The experiment was conducted for a total of 13 wks. All animals were weighed at 0800 h for 2 consecutive days at the beginning (d -35) and end of the experiment (d 56), with intermediate BW obtained on d -16 between the two vaccinations and weekly from d 0 until the end of the study. Individual feed intake and feeding behavior were registered daily throughout the study. A digital video camera (Panasonic WVCP474, Mississauga, Canada) was set up in each pen to record behavior of the animals during the first week (d 0 to d 7) in their home pen (from 0730 to 18:30). Digital video recordings were summarized by scan-sampling at 10- min intervals for standing and lying postures to

document that number of animals in each posture per pen (Mitlohner et al., 2001). A mean daily percentage of each posture was calculated. In addition, the number of animals performing active behaviors related to pain and sexual and aggressive behavior (Table 1) for a 2 min period within the 10 min scan sampling interval were recorded in each pen based on Molony et al. (1995), Thüer et al. (2007), and Mach et al. (2009). The percentage of active behaviors was transformed by cosine to achieve normal distribution. The visual analog score (VAS) was measured on d -35 and d 0 at the moment that vaccinations or band castration were performed. The observer used a VAS to document behavioral responses indicative of pain and discomfort at the time of castration. The VAS was a 10-cm horizontal line, with the far left indicating no pain response and the far right representing an extreme pain response. The observer placed a mark along this continuum that represents the amount of pain response an animal is exhibiting. The distance from the end point to the mark was measured to the nearest 0.5 cm and will be their response to the castration (Ludington and Dexter, 1998).

Table 1. Description of postures, active behaviors related to pain, and sexual and aggressive behavior of calves potentially affected by castration and recorded during the experiment.

Postures	
Standing	Standing eating, walking and playing with no obvious abnormality
Lying	Ventral or lateral recumbency with the head up or down
Active behavior related to pain	
Eating	Number of times a calf eating
Foot stamping	Hind limb lifted and forcefully placed on the ground or kicked against the abdomen while the animal was standing or lying
Tail wagging	Tail movement from side to side
Head turning	Movement of the head turned to a point on the body beyond the shoulder, e.g. scratching and grooming the testes
Grooming	Licking itself apart from the androgenital area
Sexual and aggressive behavior	
Mounting	Animal lifts its forelegs off the ground and rests the chest on the body of another animal. A mount can be on the rear, head, or side of an animal
Attempt to mount	Head on the back of other animal with the intention to mount
Head butt	Violent contact of the head on other animal body
Fighting	This can begin as head pushing but both animals brace their bodies, often resulting in the animals pushing each other off-balance or across the ground
Horning	Animals head against head without pushing
Social Licking	Licking on another animal body apart form the androgenital area
Displacements	From feeders and water point among animals

Saliva samples were collected to evaluate cortisol concentrations prior to (d -36, -16 and d -1) and the day of immunocastration and band castration (d -35 and d 0) as well as at -30, 0, 30, 60, 90, 120 and 270 min pre- and post immunocastration or band castration at d 0. Saliva samples were also obtained on d 7, 14, 21, 28, 35, 42, 49 and 56 of the study. Saliva samples were also collected according to the same schedule in the control (bull) calves. All saliva samples were obtained with a cotton swab and immediately frozen at -20ºC for later cortisol analysis as described by Cook and Schaefer (2002). A blood sample (6 mL) was obtained on d -35, -16, 0, 7, 14, 21, 28, 35, 42, 49 and 56 of the study from all calves by jugular venipuncture. Samples were collected into tubes

containing EDTA to inhibit clotting (BD Vacutainer®, Franklin Lakes, NJ) for blood count cell analysis using a Hema True Hematology Analyzer (Heska, Loveland, Colorado).

Rectal temperature was obtained from all calves using a digital thermometer (Veterinary digital thermometer, Brannan) on d -35, -16, 0, 1, 2, 5, and 7, 14, 21, 28, 35, 42, 49 and 56 of the study. In addition, thermographic images of the scrotal area were taken using a Flir i40 infrared camera and processed with ThermCam QuickView 1.3 (Flir systems Inc., Burlington, ON, Canada) to identify potential changes in blood flow and or inflammation at the scrotal site on d -35, -16, 0, 1, 2, 5, and 7, 14, 21, 28, 35, 42, 49 and 56 of the study. Lesions at the castration site were visually scored every 14 d from the time of castration (d 0) until the end of the study using an 11-point scale as previously described by Molony et al. (1995). Lesion scoring was 0: no swelling, inflammation or infection visible; 0.5-2: increasing degrees of swelling without obvious erythema; 2.5 and 3.0: swelling with obvious erythema but without pus; 3.5-5: presence of pus with increasing inflammatory response. The number of days between castration and the complete sloughing of the testes in castrated animals was also recorded. Scrotal circumference was measured on all bulls and vaccinated calves every 14 d from the time of castration until the end of the study. Scrotal circumference was measured using a metallic scrotal tape. Hair samples (250 mg) were obtained from the forehead of each animal with clippers on d -35, 0, 28, and 56 of the study. Hair samples were obtained to assess chronic stress and were stored in bags until cortisol analysis (Koren et al., 2002). After a wash with isopropanol, hair samples were ground with a ball mill for 5 min at 22 rps, sonicated with methanol for 30 min, and incubated on a shaker for 18 h, at 50 ºC and 100 rpm. The supernatant was pipette off and evaporated in a block heater, at 45 ºC under

a stream of nitrogen. Samples were were reconstituted with phosphate buffered saline before quantification of cortisol following Cook and Schaefer (2002).

On d 56 of the study, animals were intravenously injected with 2 IU porcine ACTH/kg $BW^{0.75}$ (Sigma-Aldrich, St Louis, Missouri). Immediately before, and 1, 2, and 4 h after ACTH injection, saliva samples were collected with a cotton swab and immediately frozen at -20ºC for subsequent cortisol analysis as described by Cook and Schaefer (2002). An additional 10-mL of blood samples were collected every 14 days by jugular venipuncture and harvested without additives (BD Vacutainer®, Franklin Lakes, NJ) for serum testosterone concentration and serum GnRH antibody titers analysis. All blood samples were centrifuged at 1500 x *g* at 4ºC for 15 min, and serum was stored at -20ºC until further analysis.

2.3. Chemical Analyses

Feed samples were analyzed for DM (24 h at 103ºC), ash (4 h at 550ºC), CP by the Kjeldahl method (AOAC, 1995), NDF according to Van Soest et al. (1991) using sodium sulfite and alpha-amylase, and fat by Soxhlet with a previous acid hydrolysis (AOAC, 1995). Serum cortisol concentration was determined using an immunoassay technique (Salimetric Assay Kit, State College, PA). The intra and interassay CV were 12.3 and 15.6%, respectively for samples containing 1.23 ng of cortisol/mL of saliva. Serum GnRH IgG antibody titers were determined by further development of dissociation enhanced lanthanide fluorescence immunoassay (DELFIA; Bonin et al., 1999; Ankelo et al., 2007) according to Amatayakul-Chantler et al. (2012). Intra- and interassay CV were 6.7 and 8.5%, respectively. Serum testosterone concentration was determined using a DIAsource Testo-Easia kit following the instructions of the manufacturer (Testo-Easia kit, DIAsource Immunoassays S.A., Nivelles, Belgium). Intra- and interassay CV were

4.85% and 7.15%, respectively.

2.4. Calculations and Statistical Analyses

Salivary and hair cortisol concentration as well as behavior describing animal postures were transformed to a log-scale, and behavior describing animal activity also was transformed to cosines to achieve a normal distribution prior to any statistical analysis. In addition, area under the curve (AUC) was calculated using the trapezoidal rule (Friend et al., 1977) and used to assess cortisol concentrations in the saliva after ACTH injection.

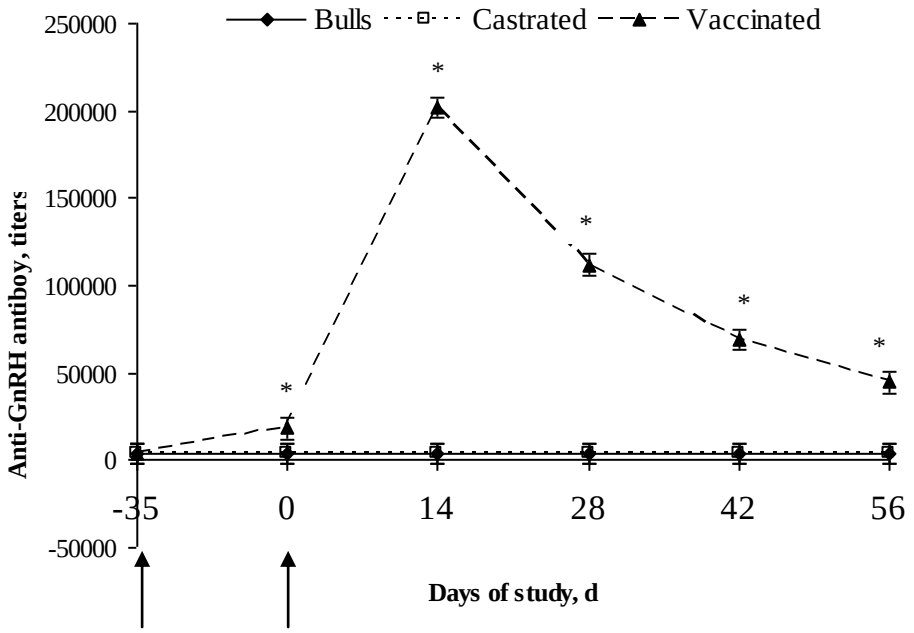
Performance, salivary and hair cortisol concentration, body and scrotal temperature and scrotal circumference data were analyzed using a mixed-effects model with repeated measures (SAS Inst. Inc., Cary, NC). The model included initial BW as a covariate, treatment, time (day), and the interaction between treatment and time, as fixed effects, and pen as a random effect. Time was considered a repeated factor, and for each analyzed variable, animal nested within treatment (the error term) was subjected to 3 variance-covariance structures: compound symmetry, autoregressive order one, and unstructured. The covariance structure that minimized Schwarz's Bayesian information criterion was considered the most desirable analysis. Behavior data were analyzed using the same model described above but pen nested within treatment. Cortisol AUC was analyzed as described above but without the time effect (as there were no repeated measures). A Chi-square-test was conducted to study the scrotal lesions and testicular consistency (categorical variables).

3. RESULTS AND DISCUSSION

3.1. Anti-GnRH antibody titers and Testosterone concentration, scrotal circumference

Serum anti-GnRH antibody titers (Figure 1) increased in vaccinated animals after the second vaccination at d 14 and remained greater ($P < 0.05$) than in bull or band-castrated calves throughout the study. These results indicate that a vaccine protocol using a 5-wk delay before revaccination had a strong IgG immune response to GnRH peptide as previously reported by Amayatakul-Chantler et al. (2012) who vaccinated *Bos indicus* x Brown Swiss bulls with the Bopriva® vaccine using a 6-wk delay before revaccination.

Figure 1. Gonadotropin-releasing hormone serum IgG titers group least square means (LSM ± SEM) of Angus and Angus crossbred bulls, band-castrated animals (castrated) or animals immunized with anti-GnRH (vaccinated) fed total mixed ration. Arrows indicate times of booster injection. Asterisks at each day point denote differences between groups ($P < 0.05$).



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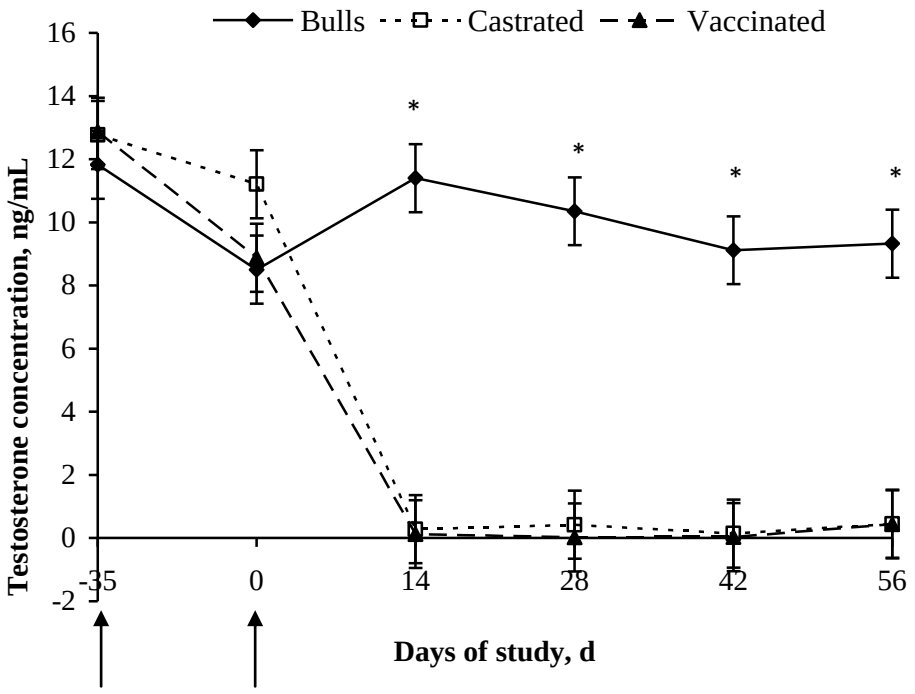
As expected serum testosterone concentration did not differ between treatments calves on d 0 (Figure 2). Serum testosterone concentration was lesser ($P < 0.05$) in band-castrated after d 0 compared to bull calves indicating that the band was effective according to (Knight et al., 2000). Serum testosterone concentration in immunocastrated calves remained below 1 ng/mL from 2 wk after the second vaccination until the end of the study because elevated antibody titers to GnRH in vaccinated animals were associated with suppression of testosterone concentration (Amayatakul-Chantler et al., 2012).

Figure 2. Serum testosterone concentration (LSM ± SEM) of Angus and Angus crossbred bulls, band-castrated animals (castrated) or animals immunized with anti-GnRH (vaccinated) fed total mixed ration. Arrows indicate times of booster injection. Asterisks at each day point denote differences between groups ($P < 0.05$).



Mean scrotal circumference measured on d -35 prior to vaccination did not differ among treatments (data not shown). However, scrotal circumference was greater ($P < 0.001$) in bulls than in immunocastrated calves on d 14 and 56 (34.6 ± 0.42 cm vs 32.8 ±

0.42 cm and 37.8 ± 0.42 cm vs 32.4 ± 0.42 cm, respectively). Similar results were observed by Hernández et al. (2005) with Nelore-cross bulls immunized against LHRH fusion proteins at 2 years of age, or Cook et al. (2000) in beef bulls immunized with an anti-GnRH vaccine at 9 mo of age.

3.2. Animal Performance and Feeding Behavior

Final BW (56 d), ADG and feed efficiency were greater ($P < 0.001$) in bulls compared to band-castrated calves, while immunocastrated calves had intermediate growth performance (Table 2). Similarly, studies conducted by Adams et al. (1996) and Aïssat et al. (2002) observed that the effect of immunocastration on ADG was intermediate between those of intact and surgically castrated animals. Some studies have reported that bulls gain more rapidly and efficiently than steers (Early and Crowe, 2002; González et al., 2010) which has been attributed to the anabolic properties of androgens, in particular testosterone (Galbraith et al., 1978, Katz, 2007). Amayatakul-Chantler et al. (2012) found that Bos indicus x Brown Swiss bulls had greater BW than animals vaccinated with anti-GnRH at 56 d post vaccination, however, by 147 d post vaccination no differences in BW was observed between the groups. In the present study the suppression of testosterone via band or immunocastration had a negative effect on calf performance (Figure 2). However, band castration was found to have a greater ($P < 0.05$) detrimental effect on animal growth than immunocastration. This may be explained by the fact that band castration is known to cause pain (Fisher et al., 1996; Pang et al., 2006**)** which can reduce feed intake as descrived later in this paper, and redirect energy required for growth to tissue repair and healing (Elsasser et al., 2008). In addition, the maintenance of a relatively high rate of growth in vaccinated animals may also be due to the residual serum concentrations of testosterone found in vaccinated animals. In contrast to the

present study, several authors observed that performance of vaccinated animals was equal to castrated animals and less than bulls (Cook et al., 2000; Ribeiro et al., 2004; Hernández et al., 2005). In addition, some studies concluded that no differences in performance were observed between vaccinated animals and intact bulls (Adams and Adams., 1992; Adams et al., 1996; Finnerty et al. 1996; Huxsoll et al., 1998; D'Occhio et al., 2001; Amayatakul-Chantler et al. 2012). However, previous studies in which the effect of immunocastration was evaluated were conducted using different vaccine types, vaccination programs (no, two or three booster doses, different time between boosters, etc) and different breeds and feeding programs compared to those used in the present study.

Table 2. Intake and performance of Angus and Angus crossbred bulls, band-castrated animals (castrated) or animals immunized with anti-GnRH (vaccinated) fed total mixed ration.

	Treatment[1]				*P*-value[2]		
Item	Bulls	Castrate	Vaccinated	SEM	T	Time	T x Time
Initial BW, kg	359	361	356	3.8	0.70	-	-
Final BW (49 d of study), kg	498[a]	456[c]	471[b]	2.9	< 0.001	-	-
ADG, kg/d	1.49[a]	1.04[c]	1.23[b]	0.047	< 0.001	0.01	0.22
Total DMI, kg/d	9.21[a]	8.73[b]	8.64[c]	0.156	0.03	0.001	< 0.01
Gain to feed ratio, kg/kg	0.16[a]	0.12[c]	0.14[b]	0.004	< 0.001	0.01	0.40

[1] Bulls = intact animals, Castrated= band-castrated animals, Vaccinated= animals vaccinated with anti-GnRH Bopriva®.
[2] T = treatment effect; Time = time effect (wk); T x Time = treatment by time interaction effect.

A significant time × treatment interaction ($P < 0.001$) was observed in feed intake. Vaccinated calves had lower ($P < 0.05$) feed intake than bulls over a 2 wk period immediately after the first and second anti-GnRH vaccinations. This was most likely due to the fact that the vaccination produced a febrile (T^{o} > 41^{o}C) response that persisted for a period of 1 wk after each vaccination. Band-castrated calves also had less ($P < 0.001$) feed intake compared with bulls. In addition, band-castrated calves had lower ($P < 0.05$) feed intake than vaccinated animals 4 wks after the second vaccination, however, no

differences in feed intake were observed between band-castrated and vaccinated animals in the last two periods of the study (from d 49 to d 56). Warnock et al. (2012) also observed a decrease in feed intake in banded animals compared to bulls. Fisher et al. (1996) suggested that the decrease in feed intake may be associated with increased pain and inflammation resulting from band castration. A treatment × time interaction ($P < 0.001$) was observed for the number of visits to the feeder as well as meal duration (data not shown); both feeder visits and meal duration decreased following band castration and vaccination resulting in the reduced feed intake as descrived above.

3.3. Acute pain response

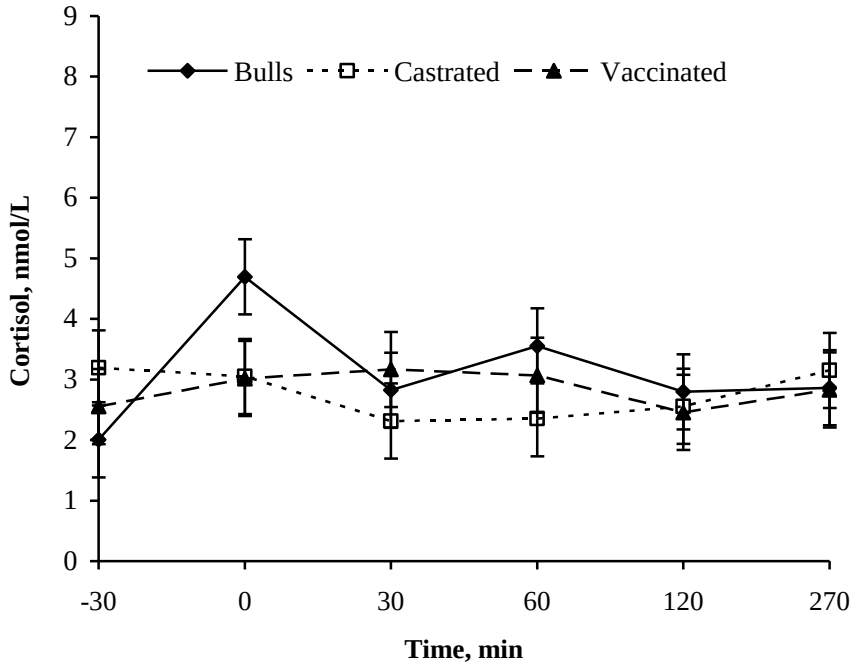
3.3.1. Salivary cortisol concentration at castration and vaccination days

On d -35, no treatment or treatment × time differences were observed in the mean salivary cortisol concentrations among treatments (3.1 ± 0.28, 2.7 ± 0.28, and 2.8 ± 0.28 nmol/L for bulls, castrated and vaccinated, respectively), which corresponded with the time that the immunocastrate group were received their first anti-GnH vaccination and the other treatment groups were administered a sham vaccination (Figure 3a).

In contrast, mean salivary cortisol concentrations were greater ($P < 0.001$) on d 0, at the time of the second vaccination and band castration, in banded (4.7 ± 0.38 nmol/L) compared to bull and vaccinated calves (2.6 ± 0.39 and 3.4 ± 0.40 nmol/L, respectively). In addition, a significant ($P < 0.001$) treatment × time interaction was observed in salivary cortisol concentration on d 0. Specifically, salivary cortisol was greater at 60 min. post vaccination in immunocastrated calves compared to the 0 min sample. However, no differences were observed between vaccinated animals and bulls after 120 min post vaccination. In addition, salivary cortisol concentration in banded calves was greater ($P <$

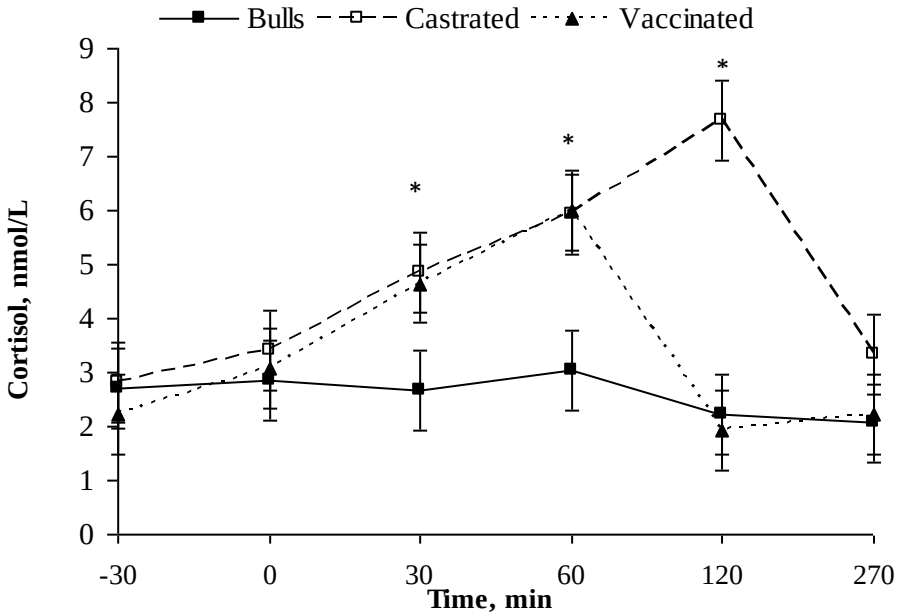
0.05) at 30, 60, and 120 min after castration compared to the 0 min sample.

Figure 3a. Evolution of serum cortisol concentration (nmol/L) of Angus and Angus crossbred of Angus and Angus crossbred bulls, band-castrated animals (castrated) or animals immunized with anti-GnRH (vaccinated) fed total mixed ration, -30, 30, 60, 120 and 270 min relative to when the procedure was performed on d -35 of the study. Asterisks at each day point denote differences between groups ($P < 0.05$).



Peak concentrations observed at 120 min were greater ($P < 0.01$) in banded calves than vaccinated calves and bulls (Figure 3b). These results indicate that the second anti-GnRH vaccination stimulated the hypothalamic-pituitary-adrenal (HPA) axis, which was not the case for the bulls that had been injected with saline solution. However, banded calves exhibited greater ($P < 0.05$) HPA axis stimulation compared to vaccinated or bull calves. Similarly, González et al. (2010) observed that band-castrated animals had increased salivary and serum cortisol concentrations, compared to bulls, indicating that pain was associated with band castration that lasted up to 4 h hours post procedure.

Figure3b. Salivary cortisol concentration (nmol/L) of Angus and Angus crossbred, band-castrated animals (castrated) or animals immunized with anti-GnRH (vaccinated) fed total mixed ration, at -30, 30, 60, 120 and 270 min relative to when the procedure was performed on d 0 of the study. Asterisk at each day point denote differences between groups ($P < 0.05$).



3.3.2. Visual analog score at castration and vaccination days

Visual analog score (VAS) indicates pain or discomfort suffered by the animals at the moment when the procedure is performed. When saline solution or anti-GnRH vaccine were injected day -35, no differences were observed in VAS among treatments (0.45 ± 0.09, 0.31 ± 0.09, and 0.34 ± 0.09 for bulls, castrated and vaccinated animals, respectively). On d 0, VAS was greater in castrated animals (2.89 ± 0.285) compared with bulls and vaccinated animals (0.63 ± 0.285 and 0.61 ± 0.285, respectively). These results are in agreement with the results of salivary cortisol concentration, indicating that band castration is a technique that produces pain or discomfort when castration is performed compared with immunocastration.

3.3.3. Behavior

Calf behavior was assessed during the first wk (from d 0 to d 7) after castration and second vaccination (Table 3). No differences in the percentage of time spent standing or lying were observed among treatments. Bulls spent 19.7 % more time ($P < 0.01$) eating than band-castrated animals. This result is consistent with the treatment difference observed for feed intake. However, although the time spent eating was numerically greater for bulls than vaccinated calves during the 1st wk the difference was not significant. Active behavior related to pain (foot stamping and tail wagging) indicated that band-castrated calves experienced more discomfort compared to bulls and vaccinated animals. The percentage of foot stamping was greater ($P < 0.01$) in band-castrated compared to bulls or vaccinated animals during the first 3 d after castration with the greatest incidence occurring on the day of castration. Thüer et al. (2007) also observed an increase of incidence of foot stamping up to 180 min after rubber-ring castration in 21-28 day old Simmental or Simental x Red Holstein calves. Active behavior related to sexual and aggressive behavior was greater ($P < 0.01$) in bulls than band-castrated and vaccinated animals. The incidence of mounting and mounting attempts was reduced from 90.9% to 86.1% in band-castrated animals, and from 74.7 % to 62.5 % in vaccinated animals. It is well known that physical castration reduces sexual and aggressive behavior of bulls (Kratz, 2007). However, an anti-GnRH vaccine appears to be equally effective in reducing aggressive and sexual behavior of bulls as observed by Jago et al. (1997) and Huxsoll et al. (1998) without compromising the welfare of the animals. It should be noted that the reduced sexual and aggressive behavior observed one wk after band castration and vaccination in the present study could also have been attributed to reduced animal activity as observed by the reduced time spent eating.

Table 3. Behavior of Angus and Angus crossbred bulls, band-castrated animals (castrated) or animals immunized with anti-GnRH (vaccinated) fed total mixed ration 1 wk after castration.

	Treatment[1]				*P*-value[2]		
Item	Bulls	Castrate	Vaccinated	SEM	T	Day	T x Day
Posture, %							
Standing	60.7	59.8	59.4	1.36	0.80	0.40	0.63
Lying	39.3	40.2	40.6	1.36	0.77	0.30	0.54
Active behavior, %							
Eating	40.6^{a}	32.6^{b}	37.8^{ab}	1.68	< 0.01	0.11	0.03
Foot Stamping	0.3^{b}	1.4^{a}	0.5^{b}	0.12	0.02	0.09	0.06
Tail waging	20.7^{b}	35.4^{a}	23.0^{b}	0.25	< 0.001	0.27	0.66
Head turning	0.22	0.31	0.16	0.17	0.70	0.09	0.92
Grooming	23.1	17.9	21.9	2.17	0.22	< 0.001	< 0.01
Mounting	1.1^{a}	0.1^{b}	0.3^{b}	0.05	< 0.001	0.13	< 0.01
Attempt to mount	0.7	0.1	0.3	0.11	0.06	0.33	0.95
Headbutt	3.0	3.1	2.4	0.15	0.77	0.53	0.91
Displacements	1.3	0.9	0.6	0.32	0.44	0.57	0.03
Fighting	0.3	1.1	0.8	0.18	0.21	0.04	0.25
Social licking	2.1	1.5	2.4	0.15	0.06	0.16	0.17
Horning	6.6^{b}	5.5^{c}	9.8^{a}	0.94	0.01	0.94	0.32

[1] Bulls = intact animals, Castrated= band-castrated animals, Vaccinated= animals vaccinated with anti-GnRH Bopriva®.
[2] T = treatment effect; Time = time effect (d); T x Time = treatment by time interaction effect.
[3] Only data corresponding 11 h were selected (7.30 am to 18.30 pm) were used to create the scan sample data set. Behavior was analyzed at scan intervals of 10 min. To represent behavior over an entire hour, scan samples were multiplied by 10. Durations (per hour) of each behavior were converted to a percentage of the total time.
[4] The values presented herein correspond to non-transformed means; however, SEM and P-values correspond to the ANOVA analyses using log- or cosines-transformed data.

3.4. Chronic pain response

3.4.1. Hair and salivary cortisol concentrations throughout the study

In recent years hair cortisol has been identified as possible indicator of chronic stress in several different species (Koren et al., 2002; Davenport et al., 2006; Accorsi et al., 2008; Comin et al., 2011; Rusell et al., 2012). It was hypothesized that, band-castrated animals in our study would have greater hair cortisol concentration at d 28 than bulls or vaccinated animals. However, no treatment or treatment × time differences in hair

cortisol concentrations were observed (81.9 ± 1.19 pg/mg, 81.3 ± 1.19 and 82.6 ± 1.20 pg/mg of hair for banded, bull and vaccinated calves, respectively). Those animals with greater salivary cortisol concentrations on d 0 (castration or second vaccination, Figure 3b) were not found to have greater hair cortisol concentrations at d 28 or d 56. In addition, lack of differences in hair cortisol concentrations throughout the study may indicate that cortisol secretion was not of significant duration or magnitude to result in a substantial deposition within the hair such that differences could be detected. These results are consistent with the other physiological indicators of chronic pain/stress measured in the present study such salivary cortisol concentration and response of ACTH injection which is described below.

A treatment × time interaction ($P < 0.001$) was observed for salivary cortisol concentration on d -35, 16, 0, 1, 2, 5, 7, 14, 28, 35, 42, 49 and 56 of the study. A rise in cortisol (From d 5 to d 14, 1.7 to 12.26 nmol/L for bulls, 1.9 to 41.9 nmol/L for band-castrated animals and 1.8 to 24.6 nmol/L for vaccinated animals) was observed for all animals on d 14 after which the concentrations returned to basal levels. This rise in salivary cortisol is unexplained, but may have been attributed the animals response to climatic conditions (snow) on that day.

3.4.2. ACTH challange

The increase in plasma cortisol concentration, as consequence of activation of the HPA axis, is one of the best known and consistent neuroendoncrine responses to stress (Sevi et al., 2002) and the aim to of administering exogenous ACTH is to stimulate adrenal secretion of cortisol to study the consequences of long-term stressors. Band castration or anti-GnRH administration did not affect salivary cortisol responses to ACTH injection compared with bulls (367.3 ± 22.80, 374.1 ± 19.44 and, 402.9 ± 24.37 nmol/L

per hour for bulls, band-castrated and vaccinated animals, respectively). According to the present study, Marti et al. (2010) did not observe differences in response to an ACTH injection in 3-mo old Holstein calves that had been ring-castrated. Mears and Brown (1997) indicated that an increase of cortisol response to stress can be attributed to relative stressfulness and the cumulative action of each stressor, however the no differences in salivary cortisol response to ACTH injection and hair cortisol observed in the present study could indicate that band castration and immunocastration do not cause chronic pain or stress.

3.4.3. Rectal temperature, Termography, Scrotal Lesion Scoring

A significant treatment × day ($P < 0.01$) interaction was observed for rectal temperature. Band-castrated animals tended ($P = 0.07$) to have greater rectal temperature on d 21 (39.3 ± 0.07, 39.5 ± 0.07, and 39.3 ± 0.07, for bulls, band-castrated, and vaccinated animals, respectively) and d 56 (39.3 ± 0.07, 39.6 ± 0.07, and 39.5 ± 0.07, for bulls, band-castrated, and vaccinated animals, respectively) than bulls. Similarly, Pang et al. (2006) observed an increase in rectal temperature in post-pubertal band-castrated animals compared to bulls. As mentioned previously, vaccinated calves had greater rectal temperature the first 7 d after vaccination (on d 1, 38.9 ± 0.07, 39.5 ± 0.07, and 38.9 ± 0.07, for bulls, band-castrated, and vaccinated animals, respectively) compared to the other treatments, which was attributed to the vaccine. At d 28 of the study, the testes of band-castrated calves began to slough off by d 28 of the study all banded calves had completely sloughed their testes by d 42 (10, 60, and 100 % of castrated animals had no testes at 28, 35 and 42 d, respectively). Similar results were observed by Pang et al. (2008) in band-castrated 12 mo old Continental, Hereford and Friesian bulls and Marti et al. (2010) in band-castrated 3 mo old Holstein calves. Mean scrotal temperature (from d 0

to d 21) was lower ($P < 0.001$) in band-castrated animals (12.7 ± 0.34 ºC) compared to bulls (27.8 ± 0.34 ºC) and vaccinated (27.6 ± 0.34 ºC) animals. These results are in agreement with the study by Marti et al. (2010) who also reported lower mean scrotal temperatures following band castration due to the blood-flow suppression after band or rings were placed. On d 14, 5% of lesion scores in band-castrated animals were classified as "1" (increasing degree of swelling without obvious erythema), and this percentage increased ($P < 0.001$) to 100% by d 28 and 95% on d 42. Pang et al. (2006) reported that shrinkage and dry necrosis in the scrotum is usually observed 2 wk following band castration. In addition, the same study found that in calves band-castrated at young ages the percentage of scrotal lesions categorized as "1" was approximately 33% 28 d after castration which is less than observed in the present study conducted using older animals. However, Molony et al. (1995) reported that band castration of calves at 1 wk of age resulted in a mean lesion score of "4" (presence of pus with increasing inflammatory response) at 28 d after castration.

3.4.4. Hematological variables

No significant treatment × day interactions were observed for percentage of WBC and RBC (Table 4). Pang et al. (2008) observed a greater RBC in band-castrated animals than bulls. However, a treatment × day interaction ($P < 0.001$) was observed in granulocyte and lymphocyte percentage as well as in blood platelet content. These hematological variables increased ($P < 0.001$) after the second vaccination in immunocastrated compared to bull and band-castrated calves. In addition, on d 21, 28, and 35 the blood platelet count of band-castrated animals was greater ($P < 0.001$) than counts in bulls and vaccinated calves, which may explain by need for tissue repair and healing associated with the sloughing of the testes. Over all, few hematological changes were observed and

most of hematological changes occurred in the first 2-3 d after castration indicating the health of the animals was not compromised 2 wks after castration or vaccination

Table 4. Total blood cell count (CBC) of Angus and Angus crossbred bulls, band-castrated animals (castrated) or animals immunized with anti-GnRH (vaccinated) fed total mixed ration.

	Treatment[1]				*P-value*[2]		
Item	Bulls	Castrate	Vaccinate	SEM	T	Day	T x Day
Granulocyte %	30.0	29.8	26.6	0.03	0.12	<0.001	<0.001
WBC count	10.5	10.2	13.2	0.15	0.42	0.44	0.33
RBC count	8.96	9.00	9.03	1.44	0.93	0.06	0.29
Lymphocyte %	62.4	63.03	32.7	1.40	0.19	<0.001	<0.001
Platelet count	330.1	355.1	327.1	12.00	0.19	<0.001	<0.001

[1] Bulls = intact animals, Castrated= band-castrated animals, Vaccinated= animals vaccinated with anti-GnRH Bopriva®.
[2] T = treatment effect; Time = time effect (wk); T x Time = treatment by time interaction effect.

4. CONCLUSIONS

In animals vaccinated with anti-GnRH vaccine, serum concentrations of anti-GnRH IgG titers increased, and in consequence serum testosterone concentration was reduced, indicating that this vaccine program was succesful. In addition, sexual and aggressive behavior during the week after castration was reduced in vaccinated animals compared with bulls. However, vaccinated calves had an increase of body temperature 7 d after injection and in consequence feed intake was reduced. Salivary cortisol concentration and visual analogue score data at castration day, and active behavior related to pain, were greater in band-castrated calves compared with bulls or vaccinated calves, indicating that these animals suffer acute pain or discomfort. In conclusion, administration of Bopriva® may be a welfare friendly alternative to traditional physical castration methods in beef calves.

5. LITERATURE CITED

Accorsi, P.A., E. Carloni, P. Valsecchi,, R. Viggiani, T. Gamberoni, C. Tamanini, and E. Seren.2008. Cortisol determination in hair and faeces from domestic cats and dogs. Gen. Comp. Endocrinol. 155:398–402.

Adams, T. E., C. A. Daley, B. M. Adams, and H. Sakurai. 1993. Testis function and feedlot performance of bulls actively immunized against gonadotropin-releasing hormone: effect of implants containing progesterone and estradiol benzoate. J. Anim. Sci. 71: 811-817.

Adams, T. E., C. A. Daley, B. M. Adams, and H. Sakurai. 1996. Testis function and feedlot performance of bulls actively immunized against gonadotropin-releasing hormone: effect of age at immunization. J. Anim. Sci. 74: 950-954.

Aïssat, D., J. M. Sosa, D. M. de Avila, K. P. Bertrand, and J. J. Reeves. 2002. Endocrine, growth, and carcass characteristics of bulls immunized against luteinizing hormone-releasing hormone fusion proteins. J. Anim. Sci. 80: 2209-2213.

Ankelo, M., A. Westerlund, K. Blomberg, M. Knip, J. Ilonen, and A. E. Hinkkanen. 2007. Time-resolved immunofluorometric dual-label assay for simultaneous detection of autoantibodies to GAD65 and IA-2 in children with type 1 diabetes. Clin. Chem. 53: 472-479.

Amatayakul-Chantler, S., J. A. Jackson, J. Stegner, V. King, L. M. S. Rubio, R. Howard, E. Lopez, and J. Walker. 2012. Immunocastration of Bos indicus x Brown Swiss bulls in a feedlot with the gonadotropin-releasing hormone vaccine Bopriva® provides imporved performance and meat quality. J. Anim. Sci. published online June 4, 2012.

AOAC. 1995. Official Methods of Analysis. 16th ed. Assoc. Offic. Anal. Chem., Arlington, VA.

Baumann, H., and Gauldie, J. 1994. The acute phase response. Immunol. Today. 15: 74-80.

.Bonin, E., M. Tiru, H. Hallander, and U. Bredberg-Raden. 1999. Evaluation of single- and dual antigen delayed fluorescence immunoassay in comparison to an ELISA

and the in vivo toxin neutralisation test for detection of diphtheria toxin antibodies. J. Immunol. Methods 230: 131-140.

Chase, C. C., Jr, R. E. Larsen, R. D. Randel, A. C. Hammond, and E. L. Adams. 1995. Plasma cortisol and white blood cell response in different breeds of bulls: comparison of two methods of castration. J. Anim. Sci. 73:975-980.

Cook, R. B., J. D. Popp, J. P. Kastelic, S. Robbins, and R. Harland. 2000. The effects of active immunization against GnRH on testicular development, feedlot performance, and carcass characteristics of beef bulls. J. Anim. Sci. 78:2778-2783.

Cook, N. J. And A. Schaefer. 2002. Stress responses of wapiti (Cervus elaphus canadensis) to removal of velvet antler. Can J. Anim. Sci. 82: 11-17.

Comin, A., A. Prandi, T. Peric, M. Corazzin, S. Dovier, and S. Bovolenta. 2011. Hair cortisol levels of dairy cows from winter housing to summer highland grazing. Livest. Sci. 138:69-73.

Davenport, M .D., S. Tiefenbacher, C. K. Lutz, M. A. Novak, and J. S. Meyer. 2006. Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. Gen. Comp. Endocrinol. 147:255–261.

D'Occhio, M. J., W. J. Aspden, and T. E. Trigg. 2001. Sustained testicular atrophy in bulls actively immunized against GnRH: potential to control carcase characteristics. 66: 47-58.

Earley, B., and M. A. Crowe. 2002. Effects of ketoprofen alone or in combination with local anesthesia during the castration of bull calves on plasma cortisol, immunological, and inflammatory responses. J. Anim. Sci. 80:1044-1052.

Elsasser, T. H., T. J. Caperna, C-J. Li, S. Kahl, and J. L. Sartin. 2008. Critical control points in the impact of the proinflammatory immune response on growth and metabolism. J. Anim. Sci. 86:E105-E125.

Finnerty, M., W. J. Enright, C. A. Morrison, and J. F. Roche. 1994. Immunization of bull calves with GnRH analogue-human serum albumin conjugate: effect of conjugate

dose, type of adjuvant and booster interval on immune, endocrine, testicular and growth responses. J. Reprod. Fertil. 101: 333-343.

Fisher, A. D., M. A. Crowe, M. E. Alonso de la Varga, and W. J. Enright. 1996. Effect of castration method and the provision of local anaesthesia on plasma cortisol, scrotal circumference, growth, and feed intake of bull calves. J. Anim. Sci. 74: 2336-2343.

Galbraith, H., D. G. Demspter, and T. B. Miller. 1978. A note on the effect of castration on the growth performance and concentration of some blood metabolites and hormones in British Friesian male cattle. Anim. Prod. 26:339-342.

González, L. A., K. S. Schwartzkopf-Genswein, N. A. Caulekett, E. Janzen, T. A. McAllister, E. Fierheller, A. L. Schaefer, D. B. Harley, J. M. Stookey, and S. Hendrick. 2010. Pain mitigation after band castration of beef calves and its effects on performance, behavior, Escherichia coli, and salivary cortisol. J. Anim. Sci. 88:802-810.

Gregory, K. E., and J. J. Ford. 1983. Effects of late castration, zeranol and breed group on growht, feed efficiency and carcass characteristics of late maturing bovine male. J. Anim. Sci. 56: 771-780.

Hernández, J. A., E. L. Zanella, R. Bogden, D. M. de Avila, C. T. Gaskins, and J. J. Reeves. 2005. Reproductive characteristics of grass-fed, luteinizing hormone-releasing hormone-immunocastrated Bos indicus bulls. J. Anim. Sci. 83: 2901-2907.

Huxsoll, C. C., E. O. Price, and T. E. Adams. 1998. Testis function, carcass traits, and aggressive behavior of beef bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 76: 1760-1766.

Katz, L. S. 2007. Sexual behavior of domesticated ruminants. Horm. Behav. 52:56-63.

Koren, L., O. Mokady, T. Karaskov, J. Klein, G. Koren, and E. Geffen. 2002.A novel method using hair for determining hormonal levels inWildlife. Anim. Behav. 63:403-406.

Knight. T. W., G. P. Cosgrove, A. F. Death, C. B. Anderson, and A. D. Fisher. 2000. Effect of method of castrating bulls on their growth rate and liveweight. New Zeland J. Agric. Res. 43: 187: 192.

Mach, N., A. Bach, C. Realini, M. Font-Furnols, A. Velarde, and M. Devant. 2009. Burdizzo pre-pubertal castration effects on performance, behaviour, carcass characteristics, and meat quality of Holstein bulls fed high-concentrate diets. Meat Sci. 81: 329-334.

Marti, S., A. Velarde, J. L. de la Torre, A. Bach, A. Aris, A. Serrano, X. Manteca, and M. Devant. 2010. Effects of ring castration with local anesthesia and analgesia in Holstein calves at 3 month of age on welfare indicators. J. Anim. Sci. 88:2789-2796.

Mears, G. J., and F. A. Brown. 1997. Cortisol and β-endorphin responses to physical and psychological stressors in lambs. Can. J. Anim. Sci. 77:689–694.

Mitlohner, F. M., J. L. Morrow-Tesch, S. C. Wilson, J. W. Dailey, and J. J. Mc Glone. Behavioral sampling techniques for feedlot cattle. J. Anim. Sci. 79:1189-1193.

Molony, V., K. E. Kent, and I. S. Robertson. 1995. Assessment of acute and chronic pain after differents methods of castration of calves. Appl. Anim. Behav. Sci. 46: 33-48.

Pang, W. Y., B. Earley. T. Sweeney, and M. A. Crowe. 2006. Effect of carprofen administration during banding or burdizzo castration of bulls on plasma cortisol, in vitro interferon-{gamma}production, acute-phase proteins, feed intake, and growth. J. Anim. Sci. 84: 351-359.

Pang, W. Y., B. Earley. V. Gath, and M. A. Crowe. 2008. Effect of banding or burdizzo castration on plasma testosterone, acute-phase proteins, scrotal circumference, growth, and helath of bulls. Livest. Sci. 117: 79-87.

Ribeiro, E. L. d. A., J. A. Hernandez, E. L. Zanella, M. Shimokomaki, S. H. Prudêncio-Ferreira, E. Youssef, H. J. S. S. Ribeiro, R. Bogden, and J. J. Reeves. 2004. Growth and carcass characteristics of pasture fed LHRH immunocastrated, castrated and intact Bos indicus bulls. Meat Sci. 68: 285-290.

Russell, E., G. Koren, M. Rieder, S. van Uum. 2012.Hair cortisol as a biological marker of chronic stress: Current status, future directions and unanswered questions. Psychoneuroendocrinology. 37: 589-601.

Sevi, A., M. Albenzio, G. Annichchiarico, M. Caroprese, R. Marino, and L. Taibi. 2002. Effects of ventilation regimen on the welfare and performance of lactating ewes in summer. J. Anim. Sci. 80: 2349-2361.

Thüer, S., S. Mellema, M. G. Doherr, B. Wechsler, K. Nuss, and A. Steiner. 2007. Effect of local anaesthesia on short- and long-term pain induced by two bloodless castration methods of calves. Vet. J. 173: 333-342.

Turner, A. S. and C. W. McIlwraith. 1989. Techniques in large animal surgery. Lea and Febiger, Philadelphia.

Vanderwert, W., L. L. Berger, F. K. McKeith, R. D. Shanks, and P. J. Bechtel. 1985. Influence of zeranol implants on growth, carcass and palatability traits in bulls and late castrates. J. Anim. Sci. 61: 537-545.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3588-3597.

Warnock, T. M., T. A. Thrift, M. Irsik, M. J. Hersom, J. V. Yelich, T. D. Maddock, G. C. Lamb, and J. D. Arthington. 2012. Effect of castration technique on beef calf performance, feed efficiency, and inflammatroy response. J. Anim. Sci. 90:2345-2352.

Chapter VI

EFFECT OF IMMUNOCASTRATION OF HOLSTEIN BULLS FED HIGH-ENERGY DIETS WITH GONADOTROPIN-RELEASING HORMONE VACCINE BOPRIVA® ON PERFORMANCE AND MEAT QUALITY

ABSTRACT

The aim of this study was to evaluate the effect of immunocastration on the performance and meat quality of Holstein bulls fed a high-concentrate diet. A total of 493 animals (weight 216-403 kg [mean 298 kg], age 200-228 days [mean 216 days] on d 0) were allocated in 4 barns with 6 pens/barn and randomized into smaller groups (pens). Each barn contained two statistical blocks (two pens/treatment), with blocking on age followed by body weight (BW). Treatment groups were intact bulls, animals surgically castrated at d 15 to 17 of the study, and animals vaccinated at d 0 and d 28 of the study with the GnRH vaccine Bopriva®. Every 14 days until d 126 of the study the BW was recorded, and blood samples were collected for serum testosterone and GnRH IgG antibody titers analysis. On d -13 to -11, 56, 84 and 126 of study testicular examination was performed. From d 131 to 133 animals were slaughtered, carcass quality performed and the 9 to 11^{th} rib section of approximately one-third of the animals randomly allocated at the start of the study were removed at 24 h post-mortem and dissected into lean, fat and bone, and meat quality was evaluated on the Longissimus muscle (LM). Elevated serum GnRH antibody titers were detected in the vaccinated group within one week post second vaccination, and maintained at a significantly higher level than those of castrated animals and bulls throughout the study ($P < 0.05$). Likewise, suppressed concentrations of serum testosterone were observed in the vaccinated group within 1 week after the second vaccination and remained significantly lower compared to the intact bulls until the end of the study ($P < 0.05$). At d 84 and 126 of the study scrotal circumference was greater in bulls than in vaccinated animals ($P < 0.05$). Testicular consistency of vaccinated animals became softer while testicular consistency of bulls became firmer with age. The average day gain (ADG) of vaccinated animals from d 0 to d 126 was intermediate ($P \leq 0.001$) between that of the ADG of bulls and the ADG of castrates. Hot carcass weight, dressing

percentage, fat classification and meat quality parameters did not differ significantly between castrated and vaccinated animals but were significantly different from those of entire bulls ($P < 0.05$). Carcass classification, pH, fat colour and loading behavior were similar in all three groups. In summary, vaccinating 7 mo of age Holstein bulls under Spanish feedlot conditions with the GnRH vaccine Bopriva® suppresses serum testosterone levels, and delivers meat and carcass quality similar to that of surgical castrates with improved ADG.

Key words: bull, feedlot, immunocastration, gonadotropin-releasing hormone vaccine, performance, meat quality

1. INTRODUCTION

Raising gonad-intact Holstein bulls fed high-concentrate diets has many advantages over conventional castrates. Intact Holstein bulls are generally more efficient in the conversion of feed into carcass (Mach et al., 2009; Marti et al., 2011a; 2011b), decreasing production costs, and improving feed efficiency compared with castrated Holstein. However, it is recognized that the management of intact animals is more difficult and adds risks to farmers (Bonneau and Enright, 1995; Jago et al., 1997). In addition, castration of Holstein bulls improves meat quality (Mach et al., 2008; Marti et al., 2011a; 2011b). Late castration, especially when performed on post-pubertal Holstein bulls fed high-concentrate diets has been a means to take advantage of greater weight gain and feed efficiency while they were bulls and the benefits of castration on meat quality characteristics thereafter (Mach et al., 2009). Alternatively, the reduction of slaughter age could be considered in order to maximize net return (Amer et al., 1994). However, when surgical castration is performed at 8 mo of age, slaughter age cannot be reduced because carcass and meat quality at 10 mo of age is impaired due to animals not fully recovering from castration sequelae (Marti et al., 2011b). Furthermore, castration requires labor and is perceived by many people to be ethically questionable (Bonneau and Enright, 1995).

One alternative to surgical castration is active immunization against gonadotrophin-releasing hormone (GnRH). Immunisation against GnRH appears to be a very attractive approach to castration of farm animals, and could potentially realize the production gains from raising entire male cattle, capture the improved meat quality, while controlling unwanted behavior by strategically timed vaccination. Pilot studies have demonstrated that, providing a good immune response is achieved, this technique is very efficient in preventing aggressive and sexual behaviour in bulls (Jago et al., 1997; Price et al., 2003).

Performance of the immunocastrated animals has been described to be equal to castrated animals and lesser to bulls (Cook et al., 2000; Ribeiro et al., 2004; Hernández, et al., 2005), intermediate between those of intact and surgically castrated animals (Adams et al., 1996; Aïssat et al., 2002;) or equal to intact animals (Adams and Adams, 1992; Adams et al., 1996; Finnerty et al., 1994 Huxsoll et al., 1998; D'Occhio et al., 2001; Amatayakul-Chantler et al., 2012). Therefore, the aim of this study was to demonstrate that immunocastration could be used as an alternative to surgical castration in 7 mo old Holstein bulls fed high-concentrate diets and evaluate its effect on performance and meat quality under Spanish husbandry.

2. MATERIALS AND METHODS

2.1. Animals, Housing, and Diets

Four hundred and ninety three Holstein calves (weight 216-403 kg (mean 298 kg), age 200-228 days (mean 216 days) on d 0) were managed following the principles and guidelines of the Animal Care Committee of IRTA (DMAH 5590 and 5591) and allocated to one of the 3 treatments: intact bulls (bulls), surgically castrated animals (castrated), and animals vaccinated with GnRH vaccine Bopriva® (vaccinated). Animals were allocated in 4 barns (Montgai, Spain) with 6 pens/barn and randomized into smaller groups (pens) of approximately 20-22 animals each. Each barn contained two statistical blocks (two pens per treatment group), with blocking firstly on age and secondly on BW. Each pen was equipped with 1 concentrate feeder providing 6 feeding spaces, 1 straw feeder providing 7 feeding spaces, and 1 drinker. All animals were fed the same concentrate (46.7% corn, 27.2% barley, 12.2% soybean meal, 7.4% soyhulls, 5.1% palm oil, 0.93% calcium carbonate, 0.3% salt, 0.2% premix; 14.6% CP, 8.7% EE, 16.0% NDF,

4.1% ash, 3.54 Mcal ME/kg; DM basis) and barley straw (3.5% CP, 1.6% EE, 70.9% NDF, and 6.1% ash; DM basis) *ad libitum* throughout the experiment.

One mL of Bopriva® (Pfizer Animal Health, Louvain-la-Neuve, Belgium) was administered to bulls in the vaccinated group on the neck through a 12.5-mm, 16-gauge needle using a safety vaccinator by single injection using a safety vaccinator on d 0 and d 28 (Simcro Safety Auto; Simcro, New Zealand) to prevent inadvertent self administration; this safety vaccinator tented the skin of the animal, facilitating administration with one hand, and ensured consistent delivery by subcutaneous injection. One mL of 0.9 % saline solution (Pfizer Animal Health, Louvain-la-Neuve, Belgium) was injected to intact bulls and surgically castrated animals on the same study days.

Surgical castration was performed on d 15, d 16 or d 17 of the study. Deep sedation was achieved through intramuscular injection (Sedaxylan®, Eurovet, Bladel, Netherland). After administration of local anesthesia, a vertical incision was made into the scrotum over the area of each testicle and through the parietal tunic to allow exteriorization of each testicle. Each testicle was removed via emasculation and division of the spermatic cord was made using an emasculator. At the same time, an analgesia (3 mg/kg BW of flunixin meglumine, Fluxinin Injectable Norbrook, Laboratorios Karizoo S.A., Spain) and antibiotic (12 mg/kg BW, procaine benzylpenicillin, Depocillin, Laboratorios Intervet, S.A., Spain) were administered i.m. Both analgesic and antibiotic treatments were repeated 48 h after castration.

2.2. Measurements and sample collection

Animals were weighed on d -14 and BW records were used to design the blocks. On d-13 to d-11, a testicle examination was performed and only animals with two

descended testicles entered the study. Animals were also weighed on d 0, d 35 and every 14 d until d 126 before animals were transported to the slaughterhouse. On d 0, d 35 and every 14 d until d 126 a 10-mL blood sample was harvested by jugular venipuncture (BD Vacutainer® SST tubes) from all animals for subsequent serum testosterone concentration and serum GnRH IgG antibody titers analysis. All blood samples were centrifuged at 1,500 x g at 4ºC for 15 min, and serum was decanted and stored at -20ºC until further analysis. On approximately d -14, d 56, 84, and 126 of study, testicular examination included an assessment of testicular consistency by manual palpation and scrotal circumference measurements using a metal scrotal tape. Consistency was graded using a 5-point scale: 1: very firm; 2: firm; 3: moderate; 4: soft; and 5: very soft.

2.3. Chemical Analyses

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

Serum GnRH IgG antibody titers were determined by further development of dissociation enhanced lanthanide fluorescence immunoassay (DELFIA; Bonin et al., 1999; Ankelo et al., 2007) according to Amatayakul-Chantler et al. (2012). Serum testosterone concentration was determined using a DIAsource Testo-Easia kit following the instructions of the manufacturer (Testo-EASIA kit, DIAsource Immunoassays S.A., Nivelles, Belgium).

2.4. Carcass and Meat Quality Measurements

On d 131, 132 or 133 animals in the study were transported to a commercial slaughterhouse (Mercabarna, Barcelona, Spain). The hot carcass weight (HCW) was recorded, and the degree of carcass fatness and conformation were graded according to the (S) EUROP categories (EU Regulation No. 1208/81, 1026/91) and into EU classification system into 1.2.3.4.5 (EU Regulation No. 1208/81), respectively. Carcass bruising score were assessed according to the Australian Carcass Bruising Scoring System (ACBSS, Anderson and Horder, 1979) and testes were collected to measure testes weight and circumference. After 4 and 26 h after slaughter, pH of LM were measured using a pH meter (PH 25 DL, Crison, Alella, Spain) on the left half of the carcass between L4 and L5. If pH at 26 h after slaughter was equal or above 5.8, carcass was defined as dark, firm, and dry (DFD).

A subsample of 56 animals for each treatment was randomly allocated at the beginning of the study to measure carcass composition. A bone-in rib section between the 9^{th} and 11^{th} ribs removed as outlined by Hankins and Howe (1946) and used to determine physical separable fat, lean, and bone and determine other meat quality parameters.

The LM was removed from each rib section, cut between the 10^{th} and 11^{th} rib and instrumental meat and fat color measurements were recorded. Lightness (L^*), redness (a^*), and yellowness (b) were measured on the exposed cut surface of the LM after 30 min of bloom time using a Minolta colorimeter (CR-400, Minolta Inc., Osaka, Japan) in the CIE-LAB space (Commission International de l'E'clairage, 1976) with illuminant D65 and 2º viewing angle. Fat thickness over rib eye and rib was measured and rib eye area was estimated using a digital image from the exposed surface and processing with image

software (Pomar et al., 2001). The LM was cut into 2 steaks (2.5 cm each) which were individually vacuum-packaged, stored at 4ºC, and then frozen after 0 and 7 d of aging for subsequent Warner-Bratzler shear force (WBSF) measurements. The remaining steak of the LM was vacuum-packaged and stored at -20ºC until determination of i.m. fat content using the Soxtec (Soxtec^{TM} 2050, Foss, Denmark) extraction method using ether petroleum as solvent (AOAC, 1995) near infrared transmission (FoodScan^{TM} analyzer, Type 78800, FOSS, Hilleroed, Denmark).

The steaks for WBSF analysis were thawed for 24 h at 2˚C, wrapped in aluminum foil and cooked to an internal temperature of 71ºC in an oven pre-heated to 200ºC. Sample internal temperature was monitored with a data logger and a thermocouple probe inserted horizontally at the steak midpoint. Cooked steaks were allowed to come to room temperature during 2 h before 6 cores (1 cm^2 cross-section x 3 cm long) were removed per steak with the fiber direction parallel to the longest dimension of the sample, and sheared perpendicular to the direction of the blade. The WBSF was measured using a texture analyzer Alliance RT/5 (MTS Systems Corp., Eden Prairie, MN, USA) equipped with a Warner-Bratzler blade with crosshead speed set at 2 mm/s.

2.5. Statistical analyses

The experimental unit was the pen. Data with repeated measures (BW, serum testosterone and anti-GnRH antibodies, testicular circumference) were log-transformed where appropriate and analyzed using a general linear mixed model for repeated measures, with terms including the fixed effects of treatment group, time point and the interactions of these effects, plus the random effects of block, pen, animal and the interaction of block, treatment group and time point. Testicular consistency scores were

summarized for each treatment group at each time point, using the number and percentage of animals in each category; the proportion of animals with a score above 2 was determined for each pen at each time point, transformed using the arcsine of the square root of the proportion, and analysed using a general linear mixed model for repeated measures, with terms including the fixed effects of treatment group, time point and the interactions of these effects, plus the random effects of block and pen. For each repeated measures model, least square means were presented for each treatment group at each time point separately, following back-transformation to the original scale if appropriate. Continuous carcass and meat quality data were analyzed using a general linear mixed model, including the fixed effect of treatment group and the random effects of block and pen. Carcass bruising, proportion of animals with DFD, and carcass classification data were summarized for each pen and treatment group using the number and percentage of animals in each category; the pen values were transformed using the arcsine of the square root of the proportion and analyzed using a general linear mixed model, including the fixed effect of treatment group and the random effect of block. For all analyses, significance was declared at $P \leq 0.05$ and tendencies were discussed at $0.05 < P \leq 0.10$.

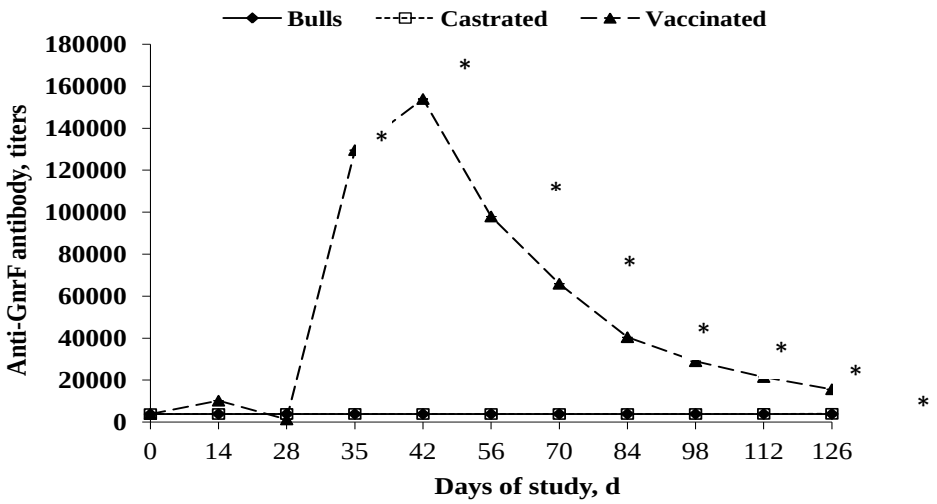
3. RESULTS

At day 0 493 animals were selected for the study but only 476 animals completed the study. Seventeen male cattle were removed. Six intact bulls were remove including 3 due to excessive mounting. Eight castrated animals including 4 related to complications due to surgery were removed. Three vaccinates none of which were related to the vaccination were removed resulting in a total of 476 animals completing the study.

3.1. GnRH and testosterone concentration, scrotal circumference

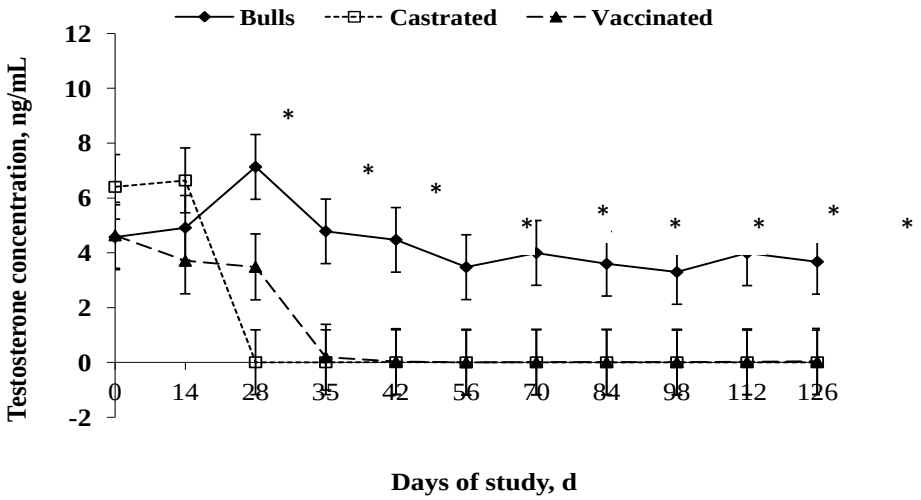
In the vaccinated group, serum GnRH antibody titers (Figure 1) increased markedly within one week after the second vaccination at d 35 and were maintained at a greater level ($P < 0.05$) throughout the study than those from castrated and entire groups

Figure 1. Gonadotropin-releasing hormone serum IgG titers group least square means (LSM ± SEM) of Holstein calves feed high concentrate diets. Asterisk at each day point denote differences between groups ($P < 0.05$). Mean titers in Vaccinated group were different to mean titers of bulls and castrated at d 35, 42, 56, 70, 84, 98, 112, and 126.



Serum testosterone on d 0 was within the range of expected for normal post-pubertal bulls (Figure 2). In the castrated group, the geometric mean serum testosterone concentration decreased ($P < 0.05$) after the castration day (d 15 to d 17 of study), and in the vaccinated group, mean serum testosterone concentration decreased ($P < 0.05$) after the second vaccination (d 28 of study), and was maintained below 1 ng/mL throughout the study.

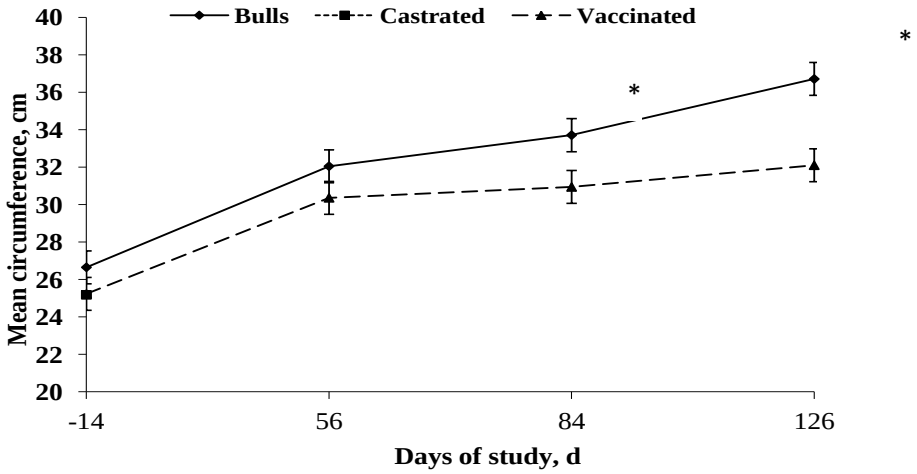
Figure 2. Serum testosterone concentration (LSM ± SEM) of Holstein calves feed high concentrate diets. Asterisk at each day point denote differences between groups ($P < 0.05$). Castrated and Vaccinated groups had reduced testosterone concentration compared with Bulls. As Castrated animals were castrated between two vaccinated days, serum testosterone concentration decreased after d 14. On d 35, Vaccinated group had suppressed testosterone concentration. Andrew, we need to check if in all day P values are < 0.05



Scrotal circumference measured prior to vaccination and castration (at approximately d -14) was not significantly different among treatments (data not shown). In Figure 3, only data corresponding to bulls and vaccinated animals are presented, as after surgical castration no more data of castrated animals were measured. At d 84 and 126 of the study, the mean scrotal circumference in entire bulls group was greater ($P < 0.05$) than in the vaccinated group. Testicular consistency of the vaccinated group became softer as the study progressed while testicular consistency of bulls became firmer ($P < 0.05$). On approximately d -14, prior to vaccination and castration, testicular consistency of the animals was mainly scored as moderate (3) or firm (2). On d 126 of the study 61.7 % of vaccinated animals had a testicular consistency scored as 4 (soft) or 5 (very soft) and only 3.7% as 2 (firm). On the contrary, testicular consistency of bulls on d 126, were

99.4% scored as 1 (very firm) or 2 (firm).

Figure 3. Mean scrotal circumference (LSM ± SEM) of Holstein calves feed high concentrate diets. Asterisk at each day point denote differences between groups (P < 0.05). Vaccinated groups had reduced scrotal circumference compared to Bulls on d 84 until the end of the study. Andrew, we need to check if in all day P values are < 0.05.



3.2. Performance

Final mean BW of the entire bulls group was greater (P < 0.05) than those of the castrated and vaccinated groups. Numerically the final BW of the castrated group was less than final BW of vaccinated group (Table 1). However, the ADG of the vaccinated group (1.43 ± 0.02 kg/d) was intermediate (P < 0.05) between the ADG of the bulls group (1.54 ± 0.02 kg/d) and the ADG of the castrated group (1.32 ± 0.02 kg/d). It is worth

noting that the statistical difference seen in the ADG between the castrated vs vaccinated group but not in the BW is due to the fact that the final average BW does not factor in the numerical difference in average group BW at the beginning of the study of 4 kg, whereas ADG does.

Table 1. Performance of Holstein bulls, bulls surgically castrated or animals vaccinated with anti-GnRH Bopriva® fed a high-concentrate diet.

	Treatment				*P-value*[1]
Item	Bulls	Castrated	Vaccinated	SEM	T
Initial age, d	216	216	216	1.66	> 0.05
Initial BW, kg	301	298	294	3.71	0.04
Final BW, kg	495[a]	465[b]	474[b]	4.12	< 0.05
ADG, kg/d	1.54[a]	1.32[c]	1.43[b]	0.020	<0.05

[1] Bulls = intact animals, Castrated= surgically castrated animals, Vaccinated= animals vaccinated with anti-GnRH Bopriva®.

3.3. Carcass and Meat Quality

Hot carcass weight ($P < 0.05$) and dressing percentage ($P < 0.05$) were greater in bulls compared with castrated and vaccinated animals (Table 2). Carcass conformation did not differ significantly among treatments; however, carcass fat cover classified as 3 was greater ($P < 0.05$) in castrated and vaccinated animals compared with bulls. Percentage of carcass bruising of bulls was reduced ($P < 0.05$) compared with castrated and vaccinated animals (Table 2).

Table 2. Carcass quality of Holstein bulls, bulls surgically castrated or animals vaccinated with anti-GnRH Bopriva® fed a high-concentrate diet.

	Treatment				*P-value*[1]
Item	Bulls	Castrated	Vaccinated	SEM	T
BW at slaughter, kg	505[a]	477[b]	485[b]	4.8	< 0.05
Hot carcass weight, kg	273[a]	252[b]	257[b]	2.5	< 0.05
Dressing percentage, %	54.1[a]	52.9[b]	53.1[b]	0.26	< 0.05
Carcass Conformation, %					
R	3.8	1.3	1.8		
O	92.4	89.1	92.7		0.33
P	3.8	9.6	5.5		
Carcass Fatness, %					
1	4.4	0.6	3.1		
2	82.3	68.6	63.6		< 0.05
3	13.3	30.8	33.3		
Bruissing, %	29.1[a]	12.2[b]	11.1[b]		< 0.05

[1] Bulls = intact animals, Castrated= surgically castrated animals, Vaccinated= animals vaccinated with anti-GnRH Bopriva®.

Moreover, meat pH at 4 h post-mortem was greater ($P < 0.05$) in bulls compared with vaccinated animals; however, the percentage of DFD carcasses was not significantly different between the three treatment groups overall. By 24 h the difference in mean pH between groups was no longer significant. Carcass fat color did not differ significantly among treatments (Table 3). Although no differences were observed in meat color luminosity, meat from bulls had less ($P < 0.05$) redness and yellowness than meat from castrated and vaccinated groups. Meat from bulls had greater ($P < 0.05$) values of WBSF at d 0 and 7 of aging when compared to meat from surgically castrated and from GnRH vaccinated groups.

Table 3. Meat quality of Holstein bulls, bulls surgically castrated or animals vaccinated with anti-GnRH Bopriva® fed a high-concentrate diet.

	Treatment				*P-value*[1]
Item	Bulls	Castrated	Vaccinated	SEM	T
Meat pH 4h post-mortem	6.47[a]	6.44[ab]	6.41[c]	0.04	< 0.05
Meat pH 26h post-mortem	5.65	5.62	5.62	0.03	0.09
DFD presence, %	10.2	3.9	5.6	0.03	0.39
Fat color[2]					
L^*	75.3	75.6	75.7	0.42	0.72
a^*	2.8	2.6	2.9	0.27	0.40
b^*	4.3	4.4	4.5	0.23	0.74
Meat color[2]					
L^*	33.7	33.9	34.0	0.32	0.81
a^*	16.1[b]	17.1[a]	16.9[a]	0.22	< 0.05
b^*	1.8[b]	2.6[a]	2.4[a]	0.16	< 0.05
WBSF at d0[3], kg	6.0[a]	5.3[b]	5.4[b]	0.31	< 0.05
WBSF at d7[3], kg	5.2[a]	4.3[b]	4.5[b]	0.24	< 0.05
Rib eye area, cm^2	70.3[a]	58.7[b]	60.4[b]	1.50	< 0.05
Fat thickness, cm	0.42[b]	0.56[a]	0.53[a]	0.032	< 0.05
Intramuscular fat, %	1.61[b]	2.28[a]	2.20[a]	0.103	< 0.05

[1] Bulls = intact animals, Castrated= surgically castrated animals, Vaccinated= animals vaccinated with anti-GnRH Bopriva®.
[2] Color: L* = lightness, a* = redness, and b* = yellowness.
[3] Warner–Bratzler shear force

As expected, the average rib eye area from bulls group was greater ($P < 0.05$) when compared with the castrated and vaccinated groups; average rib eye area from castrated and vaccinated groups did not differ significantly. However, rib eye fat thickness was greater ($P < 0.05$) in castrated and vaccinated groups than in the bulls group. Similarly, a higher percentage of i.m. fat was also observed in the castrated and vaccinated groups ($P < 0.05$) when compared to the bulls group.

Finally, rib, bone, LM and lean (without LM) weight were all greater ($P < 0.05$) in bulls than castrated and vaccinated animals (Table 4). However, according with carcass fatness classification, surgically castrated and vaccinated animals had greater s.c fat ($P <$

0.05) and intermuscular fat (P < 0.06) weight than bulls.

Table 4. Rib dissection in bone, fat and lean of Holstein bulls, bulls surgically castrated, or animals vaccinated with anti-GnRH Bopriva® fed a high-concentrate diet.

	Treatment				*P-value*[1]
Item	Bulls	Castrated	Vaccinated	SEM	T
Rib weight, g	3952[a]	3565[b]	3667[b]	81.3	< 0.05
Bone, g	783.1[a]	719.9[b]	728.4[b]	15.05	< 0.05
Fat, g					
Subcutaneous fat, g	178.3[a]	231.0[b]	226.9[b]	9.62	< 0.05
Internal fat, g	145.9	153.6	159.2	5.18	0.22
Intermuscular fat, g	394.9	444.6	450.7	17.66	0.06
Lean, g					
LM muscle, g	1193.7[a]	979.4[b]	1002.0[b]	26.24	< 0.05
Lean without LM, g	1241.9[a]	1072.2[b]	1101.2[b]	40.16	<0.05

[1] Bulls = non-castrated animals, Castrated= surgically castrated animals, Vaccinated= animals vaccinated with anti-GnRH Bopriva®.

4. DISCUSSION

Holstein bulls immunized with GnRH vaccine Bopriva® on d 0 and 28 showed strong IgG immune responses to GnRH peptide, which were sustained at elevated titers for 14 wk post second vaccination. Antibody titers to GnRH in vaccinated animals were associated with suppressed serum testosterone concentrations within 1 wk of the second vaccination. Because active immunization against GnRH results in temporary suppression of reproductive functions, it is important to determine that the vaccination program used in this study was effective at maintaining suppression of the reproductive function for the whole duration of the study ie fitting with Spanish husbandry. The results from this study confirm that the efficacy of the Bopriva® vaccine used under these conditions suppressed

serum testosterone levels similarly to those observed by Amatayakul-Chantler et al. (2012). Other reports using different vaccine formulations required either 3 doses of a GnRH (Cook et al., 2000) or the use of Freund's oil adjuvant (Adams et al., 1993) to achieve an extended duration of immunocastration effect. Decreased scrotal circumferences and testes weight observed in the vaccinated animals are consistent with those previously reported (Huxsoll et al., 1998; Cook et al., 2000; Aïssat et al. 2002; Hernández et al., 2005), supporting that the vaccination program suppressed reproductive function. The success of the present vaccination program is in accordance to Adams et al. (1996) in suggesting that 7 mo of age may be the optimal age for immunization against GnRH to generate maximal antibody production and most pronounced suppression of testicular weight in *Bos taurus* bulls, and Hernández et al. (2005) that indicated that for maximal suppression of reproduction, the final booster injection should be delivered up to 90 d before slaughter.

As expected, suppression of testosterone concentration after surgical castration was observed at the next bleed after castration indicating successful castration. However, surgical castration is associated with infections and bleeding (Turner and McIlwraith, 1989) and in some cases with the death of the animal (Gregory and Ford, 1983; Vanderwert et al., 1985), as was observed in the present study where 4 animals died within a few days after surgery due to complications.

In the present study, 3 bulls from the entire bulls group were removed from the pen due to the injuries due to excessive mounting, head butts or other aggressive behaviors. In contrast, no animals from the surgical castration or immunocastration groups were removed for these reasons, demonstrating that castration (Mellor et al., 1991; Molony et

al., 1995; Katz, 2007) and immunocastration (Jago et al., 1997; Price et al., 2003) reduces sexual and aggressive behavior of bulls.

Immunocastration is attractive if the neutralization of GnRH activity results in reduced testosterone to concentrations where sexual and aggressive behavior reduction is observed, and meat quality is improved, but at which the anabolic advantages of the normal male may still be maintained. The maintenance of a higher rate of growth in immunized bulls when compared to surgical castrates may be due to the residual serum concentrations of testosterone noted in immunized cattle. Another point to consider is setback associated with the surgical castration procedure itself that would lead to lower performance in the castrated group. Finnerty et al. (1996) have demonstrated that dose of conjugate, type of adjuvant, and interval between primary and booster injections affected antibody titres. In the literature, studies that evaluate the effect of immunocastration on performance have been conducted under very different vaccines types, vaccination programs (one, two or three booster doses, different duration of effect from booster-slaughter date) and in different breeds and husbandry. Reviewing studies were immunocastration has been effective (based on serum GnRH antibody titers), performance of the immunocastrated animals has been described to be equal to castrated animals and lesser to bulls (Cook et al., 2000; Ribeiro et al., 2004; Hernández, et al., 2005), intermediate between those of intact and surgically castrated animals (Adams et al., 1996; Aïssat et al., 2002;) or equal to intact animals (Adams and Adams, 1992; Finnerty et al., 1996; Huxsoll et al., 1998; D'Occhio et al., 2001;Amatayakul-Chantler et al., 2012). In the present study, ADG of vaccinated animals is better than ADG of castrated animals and worse than ADG of bulls. As mentioned previously, it is generally accepted that the anabolic properties of testosterone includes the promotion of muscular

development (Galbraith et al., 1978; van Tienhoven, 1983); the residual serum testosterone levels of the vaccinated animals in the present study (lower than 1 ng/mL) seem to be sufficient to improve ADG compared to castrated animals as was also reported by Aïssat et al. (2002). In addition, castrated animals suffer the stress and pain associated with physical castration (Fisher et al., 1996; Pang et al., 2006) which may impair their growth as observed in the reduction of ADG after castration. It is also worth noting that while ADG is significantly improved in GnRH vaccinated groups when compared to surgically castrated, the final BW was not. This was because the animals were allotted based on age then weight and so at d 0, the average weight was 4 kg and 7 kg less in the vaccinated group when compared to the surgically castrated and entire bull groups, respectively. Hot carcass weight follows the same pattern of the final average BW in each group. As expected, bulls had greater carcass weights and better dressing percentages than immunocastrated or castrated bulls. The decreased carcass dressing percentage observed in the vaccinated and castrated animals compared with bulls can be explained by the removal of the excess of fat in the kidney, heart, and pelvis in these carcasses with more fatcover. Previous reports have also confirmed the findings that carcass dressing percentage of bulls was greater than of vaccinated animals (Huxsoll et al., 1998), and the dressing percentage of vaccinated animals was similar to castrated animals (Adams et al., 1992; Adams et al., 1996; Huxsoll et al., 1998; Ribeiro et al., 2004). However, in contrast to the present study, some have reported no difference in carcass dressing percentage between bulls and vaccinated animals (Adams et al., 1992; Ribeiro et al., 2004; Amatayakul-Chantler et al., 2012). It is important to note that cattle breeds, husbandry practice and type of feed may contribute to the difference in performance in addition to the vaccine itself.

Reports demonstrating that entire bulls have 58.1 and 61.8 % more incidence of

carcass bruising, greater meat pH at 4 h post-mortem and greater incidence of DFD meat, greater sexual and aggressive behavior observed (Katz, 2007) compared with castrated or immunocastrated groups (Jago et al., 1997; Huxsoll et al., 1998; Price et al., 2003) may explain the carcass quality problems observed in bulls carcasses.

In the present study, the benefits on meat quality achieved by castration (carcass fat-cover, i.m. fat, tenderness score, pH) were achieved by the vaccination protocol used. The degree of marbling and other meat quality characteristics are likely related to the extent to which testicular function is suppressed and serum testosterone concentrations achieved in immunized bulls. Other studies have also observed that animals vaccinated with GnRH vaccine achieved the carcass and meat quality of castrated animals (Huxsoll et al., 1998; Aïssat et al., 2002). Amatayakul-Chantler et al. (2012), also observed that meat quality of *Bos indicus* x Brown Swiss can be improved by the use of the GnRH vaccine Bopriva® to increase the proportion of carcasses grading as USDA Choice, loin fat cover at the 12^{th} rib and meat tenderness. Cook et al. (2000) did not observe an improvement in meat tenderness when bulls were vaccinated with another GnRH vaccine; however concentration serum testosterone of the immunized bulls had a positive linear regression with shear force, suggesting that the greater the response to GnRH immunization, the more tender the meat. Similar to Ribeiro et al. (2004), rib dissection data indicate that carcass composition of vaccinated animals and castrated animals had more fat and less muscle than carcasses of bulls.

In summary, vaccinating Holstein bulls of 7 mo of age with a GnRH vaccine with a 4 wk interval between doses has successfully demonstrated suppressed serum testosterone levels for 100 d. Performance of the vaccinated group is intermediate between those from the bulls and castrated groups, but is a welfare friendly alternative to achieving the same meat quality as surgically castrated male cattle.

5. LITERATURE CITED

Adams, T. E., and M. Adams. 1992. Feedlot performance of steers and bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 70:1691-1698.

Adams, T. E., C. A. Daley, B. M. Adams, and H. Sakurai. 1993. Testis function and feedlot performance of bulls actively immunized against gonadotropin-releasing hormone: effect of implants containing progesterone and estradiol benzoate. J. Anim. Sci. 71: 811-817.

Adams, T. E., C. A. Daley, B. M. Adams, and H. Sakurai. 1996. Testis function and feedlot performance of bulls actively immunized against gonadotropin-releasing hormone: effect of age at immunization. J. Anim. Sci. 74: 950-954.

Anderson, B., and J. C. Horder. 1979. The Australian Carcass Bruises Scoring System. Queensland Agri. J. 105: 281-287

Aïssat, D., J. M. Sosa, D. M. de Avila, K. P. Bertrand, and J. J. Reeves. 2002. Endocrine, growth, and carcass characteristics of bulls immunized against luteinizing hormone-releasing hormone fusion proteins. J. Anim. Sci. 80: 2209-2213.

Ankelo, M., A. Westerlund, K. Blomberg, M. Knip, J. Ilonen, and A. E. Hinkkanen. 2007. Time-resolved immunofluorometric dual-label assay for simultaneous detection of autoantibodies to GAD65 and IA-2 in children with type 1 diabetes. Clin. Chem. 53: 472-479.

Amatayakul-Chantler, S., J. A. Jackson, J. Stegner, V. King, L. M. S. Rubio, R. Howard, E. Lopez, and J. Walker. 2012. Immunocastration of *Bos indicus* x Brown Swiss bulls in a feedlot with the gonadotropin-releasing hormone vaccine Bopriva® provides imporved performance and meat quality. J. Anim. Sci. published online June 4, 2012.

Amer P. R., R. A. Kemp, J. G. Buchanan-Smith, G. C. Fox, and C. Smith. 1994. A biometric model for comparing beef cattle genotypes at their optimal economic slaughter end point. J. Anim. Sci. 72: 38-50.

AOAC. 1995. Oficial Methods of Analysis. 16th ed. Association of Official Analytical Chemist, Arlington, VA.

Bonin, E., M. Tiru, H. Hallander, and U. Bredberg-Raden. 1999. Evaluation of single- and dual antigen delayed fluorescence immunoassay in comparison to an ELISA and the in vivo toxin neutralisation test for detection of diphtheria toxin antibodies. J. Immunol. Methods 230: 131-140.

Bonneau, M., and W. Enright. 1995. Immunocastration in cattle and pigs. Livest. Prod. Sci. 42: 193-200.

Cook, R. B., J. D. Popp, J. P. Kastelic, S. Robbins, and R. Harland. 2000. The effects of active immunization against GnRH on testicular development, feedlot performance, and carcass characteristics of beef bulls. J. Anim. Sci. 78:2778-2783.

Commission Internationale de l'E´ clairage (1976). Colorimetry. Publication nº_15, Bureau Central de la CIE, Vienna, Austria.

Field, R. A., 1971. Effect of castration on meat quality and quantity. J. Anim. Sci. 32:849-858.

D'Occhio, M. J., W. J. Aspden, and T. E. Trigg. 2001. Sustained testicular atrophy in bulls actively immunized against GnRH: potential to control carcase characteristics. 66: 47-58.

Finnerty, M., W. J. Enright, C. A. Morrison, and J. F. Roche. 1994. Immunization of bull calves with GnRH analogue-human serum albumin conjugate: effect of conjugate dose, type of adjuvant and booster interval on immune, endocrine, testicular and growth responses. J. Reprod. Fertil. 101: 333-343.

Fisher, A. D., M. A. Crowe, M. E. Alonso de la Varga, and W. J. Enright. 1996. Effect of castration method and the provision of local anesthesia on plasma cortisol, scrotal circumference, growth, and feed intake of bull calves. J. Anim. Sci. 74:2336–2343.

Galbraith, H., D. G. Demspter, and T. B. Miller. 1978. A note on the effect of castration on the growth performance and concentration of some blood metabolites and hormones in British Friesian male cattle. Anim. Prod. 26:339-342.

Gregory, K. E., and J. J. Ford. 1983. Effects of late castration, zeranol and breed group on growht, feed efficiency and carcass characteristics of late maturing bovine male. J. Anim. Sci. 56: 771-780.

Hankins, O. G., and P. E. Howe. 1946. Estimation of the composition of beef carcasses and cuts. Tech. Bull. 926:1-19.

Hernández, J. A., E. L. Zanella, R. Bogden, D. M. de Avila, C. T. Gaskins, and J. J. Reeves. 2005. Reproductive characteristics of grass-fed, luteinizing hormone-releasing hormone-immunocastrated Bos indicus bulls. J. Anim. Sci. 83: 2901-2907.

Huxsoll, C. C., E. O. Price, and T. E. Adams. 1998. Testis function, carcass traits, and aggressive behavior of beef bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 76: 1760-1766.

Jago, J. G., J. J. Bass, and L. R. Matthews, 1997. Evaluation of a vaccine to control bull behavior. Proc. N. Z. Soc. Anim. Prod. 57: 91.

Katz, L. S. 2007. Sexual behavior of domesticated ruminants. Horm. Behav. 52:56-63.

Mach, N., A. Bach, A. Velarde, and M. Devant. 2008. Association between animal, transportation, slaughterhouse practices, and meat pH in beef. Meat Sci. 78:232-238.

Mach, N., A. Bach, C. E. Realini, M. Font i Furnols, A. Velarde, and M. Devant. 2009. Burdizzo pre-pubertal castration effects on performance, behavior, carcass characteristics, and meat quality of Holstein bulls fed high-concentrate diets. Meat Sci. 81:329-334.

Marti, S., C. E. Realini, A. Bach, M. Pérez-Juan, and M. Devant. 2011a. Effect of vitamin A restriction on performance and meat quality in finishing Holstein bulls and steers. Meat Sci. 89: 412-418.

Marti, S., C.E. Realini, A. Bach, M. Perez-Juan, M. Devant. 2011b. Effect of slaughter age and castration age on performance and meat quality and carcass in high-concentrate diet fed Holstein calves. J. Dairy Sci. 94 (Suppl. 1). 490.

Mellor, D. J., V. Molony, I. S. Robertson. 1991. Effects of castration on behavior and plasma cortisol concentrations in young lambs, kids and calves. Res. Vet. Sci. 51:149-154.

Molony, V., K. E. Kent, and I. S. Robertson. 1995. Assessment of acute and chronic pain after differents methods of castration of calves. Appl. Anim. Behav. Sci. 46: 33-48.

Pang, W. Y., B. Earley. T. Sweeney, and M. A. Crowe. 2006. Effect of carprofen administration during banding or burdizzo castration of bulls on plasma cortisol, in vitro interferon-{gamma}production, acute-phase proteins, feed intake, and growth. J. Anim. Sci. 84: 351-359.

Pomar, C., J. Rivest, P. dit Bailleul, and M. Mercaux. 2001. Predicting loin-eye are from ultrasound and grading probe measurements of fat and muscle depth in pork carcasses. Can. J. Anim. Sci. 8: 429-434.

Price, E., T. Adams, C. Huxsoll, and R. Borgwardt. 2003. Aggressive behavior is reduced in bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 81: 411-415.

Ribeiro, E. L. d. A., J. A. Hernandez, E. L. Zanella, M. Shimokomaki, S. H. Prudêncio-Ferreira, E. Youssef, H. J. S. S. Ribeiro, R. Bogden, and J. J. Reeves. 2004. Growth and carcass characteristics of pasture fed LHRH immunocastrated, castrated and intact Bos indicus bulls. Meat Sci. 68: 285-290.

Turner, A. S. and C. W. McIlwraith. 1989. Techniques in large animal surgery. Lea and Febiger, Philadelphia.

Vanderwert, W., L. L. Berger, F. K. McKeith, R. D. Shanks, and P. J. Bechtel. 1985. Influence of zeranol implants on growth, carcass and palatability traits in bulls and late castrates. J. Anim. Sci. 61: 537-545.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3588-3597.

van Tienhoven, A. 1983. Reproductive physilogy of vertebrates. Cornell University Press, New York.

Chapter VII

GENERAL DISCUSSION

In the past, Spanish beef industry was focused to produce the maximum quantity of meat even if the meat quality produced did not present the maximum quality. However, nowadays, with the Welfare state, consumer starts to demand more quality in the products. In addition, globalization permits importation of meat from other countries with lower production costs and similar meat quality. For these reasons, beef producers have more concerns to produce the maximum quantity with the maximum quality. Results form Chapter IV and VI presented castration as a good strategy to improve meat quality. Castration improved the carcass fat cover, and also improved i.m. fat, one of the most important meat quality parameters, juiciness, flavor, meat color and tenderness. However, castration is considered one of the most painful practice in animals and is questioned from the welfare point of view, although is a common practice in a lot of countries. Chapter III evaluated the effect of ring castration at 3 mo of age on welfare indicators; this technique and age, also the use of anesthesia and analgesia, were chosen to reduce the pain produced by the castration. The main goal of this early castration was to achieve a good meat quality (fat cover, i.m. fat) in Holstein bulls at early ages. The results of Chapter III indicated that pain produced by castration was reduced but not totally eliminated until 14 d post-castration. For these reason, Chapter V evaluated an alternative method to physical castration based on the use of a vaccine against GnRH. Results form Chapter V showed that vaccine against GnRH reduced testosterone levels below 5 ng/mL at the same extend to physical castration; also reduced salivary cortisol concentration and active behavior related to pain compared with band-castrated animals. In addition, sexual and aggressive behavior was reduced compared with bulls as observed in castrated animals. Therefore, in Chapter VI, where performance and meat quality was evaluated in animals vaccinated against GnRH, again in vaccinated animals a reduction in serum testosterone concentration was observed, and these animals produced meat with the same meat quality

as surgically castrated animals. However, as described in Chapter V, vaccinated animals had an increased rectal temperature during 1 wk after injection, but overall performance in vaccinated animals did not decrease compared with physical castrated animals because vaccinated animals did not suffer the detrimental effects of pain derivered from the mutilation indicated by salivary cortisol concentration and behavior traits compared to the band-castrated animals.

The effect of physical castration and immunocastration will be discussed in two separate sections: castration and welfare, and castration and meat quality.

1. CASTRATION AND WELFARE

Chapter III and Chapter V the welfare of animals castrated with different methods (rubber-rings, bands and vaccine against-GnRH) was evaluated. However, breeds, age at castration, castration techniques and methods of pain mitigation were different among the studies.

Castration reduces overall animal performance, however, it would be interesting to clarify if this performance reduction is caused by the reduction of intake due to pain produced by castration, or if it is because the lack of testosterone or both. Animals castrated at 3 mo of age with rubber-ring, at 8 mo of age with surgical castration, at 8 mo of age with bands or vaccinated against GnRH reduced their overall ADG compared with bulls. However, the main ADG reduction was around the castration (Figures 1, 2, 3, and 4), the welfare indicators evaluated in Chapter III and Chapter IV support this hypotheses as they indicate that animals suffered discomfort during 2 wks after castration. In addition, in most cases the ADG of castrated animals was below the ADG of bulls throughtout the experimental period, so part of the weight losses in castrated animals could be also due to reduction of testosterone concentration. In vaccinated animals, the

fever during the first 7 d post-vaccination could be the reason by animals decreased their intake and ADG, however animals recovered quickly their ADG (Figures 1, 2, 3, and 4). The lack of discomfort signs, the reduced serum testosterone concentration and ADG in vaccinated animals compared with bulls would support the hypothesis that serum testosterone concentration is positively correlated to ADG.

Figure 1. Average daily gain of Holstein bulls and 3 mo old ring-castrated animals fed high-concentrate diets of Chapter III.

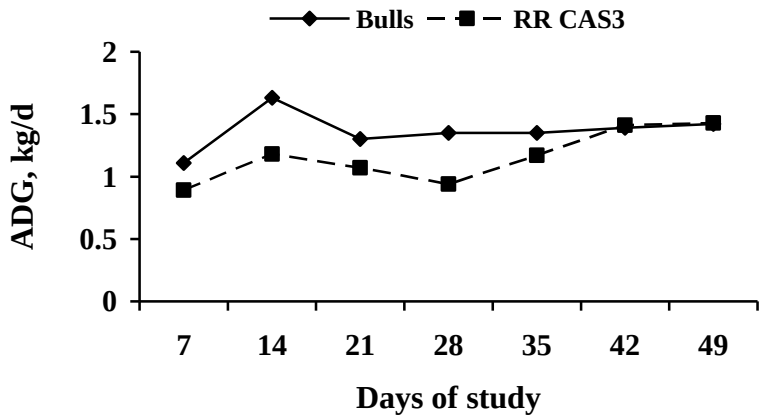


Figure 2. Average daily gain of Holstein bulls, 3 month old ring-castrated animals, 8 month old surgically castrated animals fed high-concentrate diets of Chapter VI.

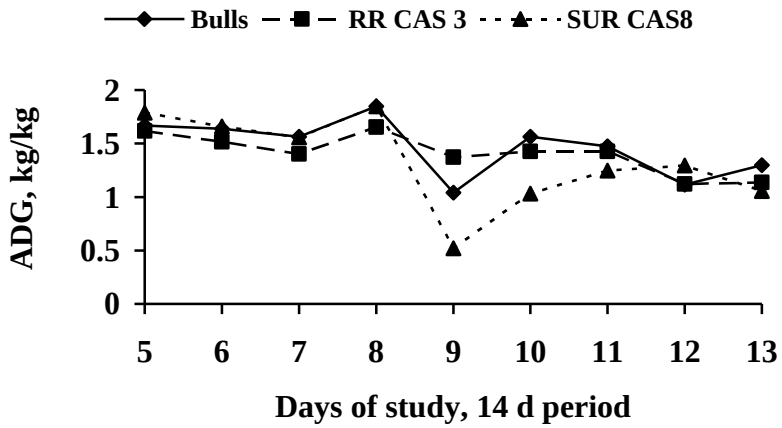


Figure 3. Average daily gain of Angus crossbred bulls, 8 month old band-castrated animals and immunocastrated animals at 8 month of age fed total mixed ration of Chapter V.

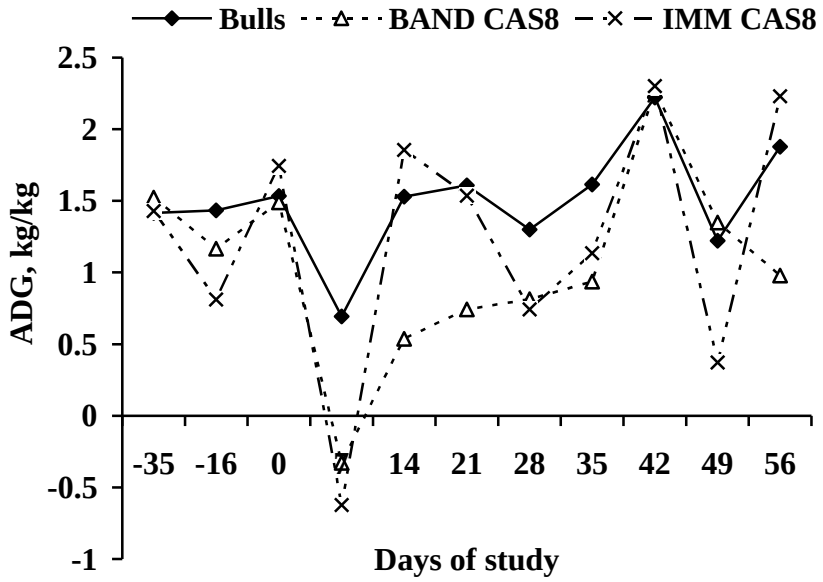
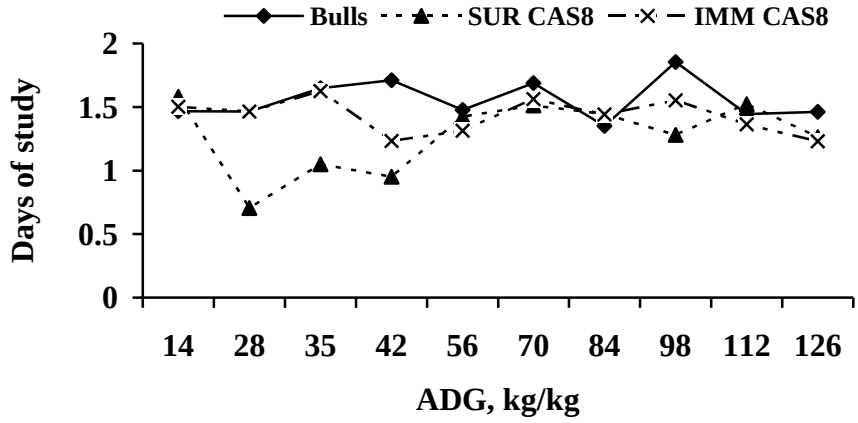


Figure 4. Average daily gain of Holstein bulls, 8 month old surgically castrated animals and immunocastrated animals at 8 month of age fed high-concentrate diets of Chapter VI.



In Chapter III, Holstein bulls were compared with animals castrated at 3 mo of age with rubber-rings using a pain mitigation procedure; in contrast, in Chapter V, Angus crossbred bulls were compared with animals castrated with bands without a pain mitigation procedure and animals vaccinated against GnRH. Castration of animals at pre-

pubertal ages causes less pain due to the less testicular development compared to post-pubertal castration (Mellor et al. 1991).

However, at 60 min post castration cortisol concentration in pre- and post-puberty castration calves was similar (about 5 mmol/L). These results could indicate that acute pain produced by castration could be not related to castration technique (ring vs. band) and age at castration (3 mo vs. 8 mo). In animals that received a pain mitigation procedure, as in Chapter III, serum cortisol concentration was reduced to 2.5 mmol/L at 180 min post castration. However, in Chapter V where those animals were castrated without a pain mitigation procedure, serum cortisol concentration increased to about 7.5 mmol/L at 120 min post castration. These results are in agreement with different authors (Fisher et al., 1996; Early and Crowe, 2002; Stafford et al., 2002; Gonzalez et al., 2010) that indicate that the use of anesthesia reduces serum cortisol concentration. The increase of serum cortisol concentration in animals vaccinated against GnRH at 60 min after the second vaccination could be related to the immune system stimulation caused by the vaccine, as indicated by the increase of rectal temperature after vaccination, and not probably this serum cortisol concentration increase was not related to a pain response (indicated by behavior data) as observed in the physical band-castrated animals. Moreover, serum cortisol concentration at 120 min post vaccination in vaccinated animals was reduced to the same levels of bulls.

In Chapters III and V behavior related to pain was evaluated. In both chapters no differences were observed in standing and lying percentages between castrated animals and bulls. No treatment by time interaction was observed when animals were castrated at 3 mo of age with rubber-ring, castrated at 8 mo of age with bands, or vaccinated animals against GnRH and bulls. However, Devant et al. (2012) using data from the same study of

Chapter IV, observed that surgical castration at 8 mo of age decreased the lying time after castration, whereas no differences between bulls and animals castrated at 3 mo of age with rubber-rings in lying time were observed. Moreover, during the study described in Chapter IV and Chapter VI, were surgical castration was performed some animals died according with Gregory and Ford, (1983) and Vanderwert et al. (1985) indicating that surgical castration may cause the death of the animals. The results of active behavior observed in Chapters III and V showed that castration produced an increase in head turning percentage in animals castrated at 3 mo of age with rubber-rings, whereas in animals castrated at 8 mo of age with bands the foot stamping and tail wagging percentage increased. Therefore, physical castration produces changes in animal behavior during the first 2 wks after castration. Moloney et al. (1995) and Thüer et al. (2007), according with the Chapter II, did not observe changes in head turning percentage when animals were castrated with in rubber-ring at young ages. In addition, Moloney et al. (1995) observed that rubber-ring-castrated animals increased the foot stamping percentage compared with other castration methods. In agreement to these authors (Moloney et al., 1995), when animals were band-castrated at older ages foot stamping increased compared with bulls or vaccinated animals. In vaccinated animals no behavior related to pain was observed being similar to bulls, indicating that no pain or discomfort was observed in animals vaccinated against GnRH.

When chronic pain was evaluated by serum haptoglobin concentration, cortisol concentration after ACTH injection and/or hair cortisol, no differences among castrated animals and bulls in the different studies were observed. It might indicate that castration with rubber-ring or bands does not produce chronic pain or stress, and it these techniques could be compared to other castration techniques like Burdizzo that only produces an

acute pain. In contrast to the present studies results, other authors that evaluate the effect of castration methods on chronic pain or stress conclude that rubber-ring or band castration are castration techniques that produce chronic pain (Moloney et al. 1995; Thüer et al. 2007). Furthermore, chronic pain parameters evaluated in animals vaccinated against GnRH were not different from those of bulls, indicating that these animals did not suffer chronic pain after vaccination. May be the techniques used to estimate chronic pain are not accurate enough.

2. MEAT QUALITY AND CASTRATION

Chapters IV and VI evaluated the effect of castration on meat quality of Holstein bulls. To compare the effect of different castration methods with the vaccine against GnRH and bulls on meat quality, only data from the animals slaughtered at 12 mo of age in Chapter IV were used to be able to compare them with the slaughter age described in Chapter VI (Table 1). The effect of the castration (physial or vaccine) on meat quality was consistent.

Table 1. Carcass and meat quality traits of Holstein calves fed high-concentrate diets

Item	Chapter IV[a]			Chapter VI[a]		
	Bulls	SUR CAS8	RR CAS3	Bulls	SUR CAS8	IMM 8M
HCW, kg	262	251	254	273	252	257
Carcass Fatness "3", %	0	38.5	60	13.3	30.8	33.3
Carcass Conformation "O", %	92.9	92.3	80	92.4	89.1	92.7
WBSF at d0, kg	6.8	6.8	5.6	6.0	5.3	5.4
WBSF at d7, kg	4.9	5.7	5.2	5.2	4.3	4.5
Intramuscular fat, %	1.66	2.30	2.74	1.61	2.28	2.20

[a]Bulls; SUR CAS8: surgical castration at 8 mo of age; RR CAS3: rubber-ring castration at 3 mo of age; IMM 8M: anti-GnRH vaccine at 8 mo of age.

Results form Chapters IV and VI demonstrated that anabolic hormones produced by testes (Adams et al. 1996) promote muscular development throughout the increase in nitrogen retention (Katz et al. 2007) as observed by the greater HCW in bulls comparesd with physical castrated or vaccinated animals. So, it is clear that suppression of serum testosterone is a good strategy to improve meat quality. Knight et al. (1999) proposed post-puberty castration to approach the benefits from the faster performance of intact males (greater growth rate and feed efficiency, and carcass weight) before castration at post-puberty ages, and approach the benefits from the effect of castration on meat quality afterwards. In contrast to these authors, as observed in Chapter IV, HCW of pre- and post-puberty castrated animals, when castration was a physical castration method, was similar. But, Knight et al. (1999) proposed strategy could be an effective strategy when castration is achieved by the vaccination against GnRH and no pain is produced, as discussed previously. As observed in Chapter VI, vaccinated animals had greater HCW than castrated animals. In addition, although no differences in HCW between pre- and post-puberty animals, the benefits obtained by the producers could be greater if castration is performed post-puberty because these animals will have a greater percentage of carcasses classified as "O". So, to benefit from the positive effect of the post-puberty castration on carcass weight and carcass conformation, vaccination against GnRH is the best alternative. Vaccinated animals against-GnRH, which reduced serum testosterone concentration below 5 ng/mL, as described by other authors (Amayatakul-Chantler et al., 2012), and achieved the same carcass conformation and fat-cover as animals castrated post-puberty with physical methods. As mentioned before, vaccination is an attractive method to reduce serum testosterone concentration because final HCW is greater than in animals castrated post-puberty with physical methods. The vaccination positive effects on

HCW could be explained by the residual serum concentration of testosterone and/or because this method did not produce pain compared to physical castration.

Another trait that determines final carcass price is carcass fat cover. The carcass fatness classification desired in Catalonia is "3". This carcass fat cover permits a better aging of the carcass, for this reason carcasses of castrated animals (physical castrated or vaccinated) could have a better acceptance in the industry than carcasses of intact males. In Chapter IV, no carcasses classified as "3" were observed in bulls and only a 13% were observed in Chapter VI. These differences in carcass fat cover between studies could be attributed to the different concentrated fed to these animals (5.4 % of EE and 3.25 Mcal/kg in Chapter IV vs. 8.7% of EE and 3.53 Mcal/kg in Chapter VI). In addition, if castration is performed at early ages, more carcass fat cover will have the animals as observed in Chapter IV in animals castrated at 3 mo of age.

Purchas et al. (2002) associated the increase in tenderness in castrated animals compared with bulls to slightly lower ultimate pH and greater i.m. fat. Chapters IV and VI are in accordance to these authors, i.m. fat of physically castrated or vaccinated animals was greater, and also the tendersness of meat of castrated animals was greater than the tenderness of bulls. Miller et al. (2001) defined the acceptance level of tenderness when WBSF were below "4", these values were only achieved on d 0 in physically castrated or vaccinated animals. Another parameter that affects tenderness is the ultimate pH. In Chapter IV and VI, intact males had greater incidence of high ultimate pH than physically castrated or vaccinated animals, and it could influence characteristics of meat (Mounier et al. 2005). The aggressive and sexual behavior of bulls, they have a more excitable temperament as observed in Chapter V, results in a greater ante-mortem glycolysis and pH (Monin, 1990), and greater incidence of carcass bruising (Chapter VI) compared to castrated animals and these results in a devaluation of carcass prices.

3. CASTRATION AND PERSONAL TOUGHTS

After 4 years studing the effect of the castration on animal welfare and performance, I still have some unsolved questions and contradictory feelings.

Some authors defined castration as one of the most painful experiences for calves because these animals suffer pain, physiologic stress, inflammatory reactions, immune response suppression and performance is reduced. However, in the studies summarized in this Thesis indicators that estimate all these alterations after castration were sometimes not altered.

When animals suffer pain, commonly it's measured by serum cortisol concentration; in the present Thesis some contradictions with bibliography were found. In chapter III mean serum cortisol concentration from 30 to 180 min was greater in bulls than in castrated animals and it was justified because in castrated animals anesthesia and analgesia was used. But in Chapter V, mean salivary cortisol concentration did not differ among treatments, although in this latest study no anesthesia or analgesia were used. Also, it's generally accepted based on the literature that post-pubertal castration causes more pain than pre-pubertal castration, then it was supposed that in Chapter V, salivary cortisol concentration should have greater in castrated animals than in bulls, and than in the castrated animals from Chapter III, and this hypothesis was not observed. In addition, if castration is one of most painful practice in calves, why climatological changes as observed between day 5 and d 14 in Chapter V had a greater impact on salivary cortisol concentration than the impact that castration had on salivary cortisol concentaration? Maybe the pain caused by castration is less than the pain expected by scientific community?

Another parameter that is used to measure pain is behavior, as observed in Chapter III and Chapter V, castration alters behavior, but the alterations in behavior disappeared after 2 wks post-castration. These changes in behavior were mainly observed in ring or band castration. Is it enough to afrim that these changes in behavior are due to pain? Or is a discomfort produced by a physiological change due to castration? When a ring or band is placed in the scortum, after 24 hours the temperature of scrotum area decreases and the blood-flow is suppressed, then animals increase active behaviors like kicking, and it is because these animals have pain or because they feel discomfort due to the presence of this ring or band? Maybe surgical castration is the only method were it is clearer that it produces pain, this affirmation is based on the pronounced decrease in ADG the weeks after castration, and on the decrease in lying time the weeks after castration. The European legislation suggests using the surgical castration in case that a castration needs to be done. May be this recommendation should be questioned, as in addition to the pain, animals suffer more infecctions and the risk to die is greater.

During this Thesis three of the most common or recently proposed methods to determine the chronic pain or stress have been used. Acute phase proteins, serum cortisol concentration after ACTH injection, behavior (beyond 2 wks after castration), and hair cortisol concentration should to be altered if castration causes chronic pain or stress in calves. However, castration applied at different ages and/or methods in the studies summarized in the present Thesis did alter none of these parameters. It's because these parameters are not the best ones to measure chronic pain or stress in calves, or is because castration does not cause chronic pain or stress? Acute phase protein or hair cortisol concentrations were altered when the effect of stress caused by animal handling (movement from winter housing to summer grazin descrived by Comin et al. (2011) or aclimmatation to human handling descrived by Francisco et al. (2012) was evaluated. If

we ask to the animal science technicians what animal practice produces more stress or pain, and they need to choose between castration and handling, practically all of them would choose castration. The human perception does not fit with the measurements that are actually used to evaluate chronic pain or stress, as these parameters were altered by animal handling, but not by castration.

Regarding meat quality, results from this Thesis and data of literature are in total agreement, castration improves meat quality. However, but if somebody decides to use castration as strategy to improve meat quality, he needs to be aware that castration impairs performance, even if castration is performed at pre- or post-pubertal ages. The negative effects of castration on performance are however reduced when immunocastration is used. In addition slaughter age and castration age may influence the benefits of the producers. Moreover, it is great iniciative that Spanish beef producers start to have the interest to improve meat quality, but in case they want to improve meat quality some support should be found to promote this strategy. First of all, meat quality should be paid. In North-America (and I'm not saying that they do all the things perfect) beef producers do not want produce bulls, and meat plants do not want bulls as well, however meat quality is paid, so the performance losses produced by castration are compensate mainly by the price based partially on meat quality (marbling). Why can the Spanish beef producers not adopt this method (prices based on meat quality) to promote a product has a better quality?

Regarding the use of immunocastration as an alternative to immunocastration, I expected that this method was a very good method to avoid the welfare problems of physical castration, to improve meat quality and reduce the performance losses caused by physical castration. However, some aspects of the vaccine should be considered. First of all, although it's applied by safety vaccinators there is the risk to get injected, with the

consequent antibody against GnRH development by the person who was injected; if a second injection is received a possible immunization could develop. Risks about it should be minimized. In addition, would be better if the number of boosters could be reduced to 1, then the risk of self-injection and labor would be reduced. Finally, a good protocol of boosters (number, time between them, etc) needs to be defined for each production system to make sure that these animals will be immunized for the period of time expected by the producer.

4. LITERATURE CITED

Adams, T. E., C. A. Daley, B. M. Adams, and H. Sakurai. 1996. Testis function and feedlot performance of bulls actively immunized against gonadotropin-releasing hormone: effect of age at immunization. J. Anim. Sci. 74: 950-954.

Amatayakul-Chantler, S., J. A. Jackson, J. Stegner, V. King, L. M. S. Rubio, R. Howard, E. Lopez, and J. Walker. 2012. Immunocastration of *Bos indicus* x Brown Swiss bulls in a feedlot with the gonadotropin-releasing hormone vaccine Bopriva provides imporved performance and meat quality. J. Anim. Sci. published online June 4, 2012.

Comin, A., A. Prandi, T. Peric, M. Corazzin, S. Dovier, and S. Bovolenta. 2011. Hair cortisol levels of dairy cows from winter housing to summer highland grazing. Livest. Sci. 138:69-73.

Devant, M., S. Marti, and A. Bach. 2012. Eating pattern of Holstein bulls and steers fed high-concentrate rations using a computerized concentrate feeder. J. Anim. Sci. Online June. http://jas.fass.org/content/early/2012/06/13/jas.2011-4929.

Earley, B., and M. A. Crowe. 2002. Effects of ketoprofen alone or in combination with local anesthesia during the castration of bull calves on plasma cortisol, immunological, and inflammatory responses. J. Anim. Sci. 80:1044-1052.

Fisher, A. D., M. A. Crowe, M. E. Alonso de la Varga, and W. J. Enright. 1996. Effect of castration method and the provision of local anesthesia on plasma cortisol, scrotal circumference, growth, and feed intake of bull calves. J. Anim. Sci. 74:2336–2343.

Francisco, C. L., R. F. Cooke, R. S. Marques, T. Leiva, F. Sanches, A. Bouck, F. N. T. Cooke, and D. W. Bohner. 2012. Effects of aclimatation to human handling on temperament, physiologic responses, and performance of beef steers during feedot receiving. Production, management and environment: Beef production. ADSA®-ASAS Joint Annual Meeting, Phoenix. Abstract 397.

González, L. A., K. S. Schwartzkopf-Genswein, N. A. Caulekett, E. Janzen, T. A. McAllister, E. Fierheller, A. L. Schaefer, D. B. Harley, J. M. Stookey, and S. Hendrick. 2010. Pain mitigation after band castration of beef calves and its effects on performance, behavior, Escherichia coli, and salivary cortisol. J. Anim. Sci. 88:802-810.

Gregory, K. E., and J. J. Ford. 1983. Effects of late castration, zeranol and breed group on growht, feed efficiency and carcass characteristics of late maturing bovine male. J. Anim. Sci. 56: 771-780.

Katz, L. S. 2007. Sexual behavior of domesticated ruminants. Horm. Behav. 52:56-63.

Knight, T. W., G. P. Cosgrove, A. F. Death, C.B. Anderson. 1999. Effect of interval from castration of bulls to slaughter on carcass characteristics and meat quality. New Zealand J. Agric. Res. 42:269-277.

Mellor, D. J., V. Molony, I. S. Robertson. 1991. Effects of castration on behavior and plasma cortisol concentrations in young lambs, kids and calves. Res. Vet. Sci. 51:149-154.

Miller, M., M. Carr, C. Ramsey, k. Crocket, and L. Hoover. 2001. Consumer thresholds for establishing the value of beef tenderness. J. Anim. Sci. 79:3062-3068.

Molony, V., K. E. Kent, and I. S. Robertson. 1995. Assessment of acute and chronic pain after differents methods of castration of calves. Appl. Anim. Behav. Sci. 46: 33-48.

Monin, C. 1990. Facteurs biologiques et qualities de la viande. In: R. G. Guilhermet and Y. Gea, Editor, Croissance des bovins et qualité de la viande, ENSA, Rennes (1990), pp. 177-196.

Mounier, L., I. Veissier, and A. Boissy. 2005. Behavior, physiology, and performance of bulls mixed at the onset of finishing to form uniform body weight groups. J. Anim. Sci. 83:1696-1704.

Purchas, R. W., D. L. Burnham, and S. T. Morris. 2002. Effect of growth potential and growth path on tenderness of beef *longissimus* muscle from bulls and steers. J. Anim. Sci. 80:3211-3221.

Stafford, K. J., D. J. Mellor, S. E. Todd, R. A. Bruce, and R. N. Ward. 2002. Effects of local anaesthesia or local anaesthesia plus a non-steroidal anti-inflammatory drug on the acute cortisol response of calves to five different methods of castration. Res. Vet. Sci. 73: 61-70.

Thüer, S., S. Mellema, M. G. Doherr, B. Wechsler, K. Nuss, and A. Steiner. 2007. Effect of local anaesthesia on short- and long-term pain induced by two bloodless castration methods of calves. Vet. J. 173: 333-342.

Vanderwert, W., L. L. Berger, F. K. McKeith, R. D. Shanks, and P. J. Bechtel. 1985. Influence of zeranol implants on growth, carcass and palatability traits in bulls and late castrates. J. Anim. Sci. 61: 537-545.

Chapter VIII

CONCLUSIONS

Studies summarized in this Thesis evaluating the effects of physical castration and immunocastration in early-maturing bulls fed high-concentrate diets on welfare indicators, performance, and carcass and meat quality allow us to conclude that in our experimental conditions:

1. **Ring castration performed at 3 mo of age with local anesthesia and analgesia in Holstein bulls decreases ADG, affects some behavioral trails during 2 wks after castration, but does not alter other welfare indicators like feed intake, serum cortisol concentration, or serum haptoglobin concentration. These results indicate that ring castration at 3 mo of age using analgesia and anesthesia can be considered a method that controls acute pain or discomfort and does not greatly compromise animal welfare.**

2. **In Holstein animals, castration age affects performance and meat pH independently of slaughter age, and slaughter age affects performance and meat pH independently of castration. However, in Holstein animals, castration affects seveal characteristics related to fat depositions differently depending on slaughter age, such as carcass fat cover, and intramuscular, intermuscular and subcutaneous fat.**

3. **Bulls castrated at 3 month of age could be slaughtered at 10 month of age as intramuscular fat and carcass fat cover would be close to the values of an intact bull slaughtered at 14 mo of age, in consequence the days on fed and the production costs could be reduced.**

4. **Surgical castration performed at 8 mo of age is not good strategy to reduce slaughter age as carcass and meat is impaired (carcass fat cover or**

intramuscular fat) because at 10 mo of age these animals have not fully recovered from surgical castration.

5. **When band castration is used in animals at 8 mo of age animals suffer acute pain or discomfort as indicated by the increase of salivary cortisol concentration and visual analog score at the day of castration, the increase in foot stamping and tail wagging seven days after castration, and the decreased ADG compared to bulls and immunocastrated animals.**

6. **The two vaccine protocols used in the studies summarized in the present Thesis are effective, as animals vaccinated with Anti-GnRH had an increase the serum anti-GnRH IgG titers and in consequence serum testosterone concentration was reduced for 56 d in the first study and for 100 days in the second study.**

7. **At the second vaccination day with Anti-GnRH animals have an increase salivary cortisol concentration compared with bulls, but this concentration is less than in band-castrated animals. Also, after the second vaccination, immunocastrated animals suffer an increase of body temperature during one week that could explain the reduction of feed consumption observed in these animals during this week. No differences in behavior related to pain are observed between immunocastrated animals and bulls.**

8. **Performance and efficiency of immunocastrated animals are intermediate between physically castrated, band-castrated or surgically castrated animals, and bulls.**

9. **Immunocastrated animals have a similar meat quality to surgically castrated**

animals.

10. **Therefore, immunocastration could be welfare friendly alternative to produce beef meat with a carcass and meat quality similar to physically castrated animals.**