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ORIGINAL ARTICLE

Effect of castration at 10 months of age on growth physiology and behavior of male feral beef cattle

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ABSTRACT

This study compared the growth performance, plasma testosterone and cortisol levels around castration at 10 months of age, and plasma insulin-like growth factor (IGF)-I concentration and flight speed, in intact bulls and steers from 10 to 21 months of age in a feral Spanish breed. Fourteen bulls (366.5 ± 48.5 kg live weight) were assigned at random to one of two treatments: surgically castrated (steers) or intact (bulls), and submitted to an identical fattening period. Steers reared until heavy live weights (21 months of age) grew slowly and had lower plasma IGF-I concentrations than intact bulls. These differences were mainly highlighted the month after surgery (11 months of age) and the last part of the fattening period (from 19 to 21 months of age). After surgical castration (11 and 12 months of age), steers showed a tendency to display greater flight speed values than intact bulls but baseline plasma cortisol concentration did not differ between groups at this time. At the end, steers and bulls reached nearly similar temperament, as flight speed did not differ between them. The results confirm the role of IGF-I as a key anabolic hormone in male beef cattle and thus it may reflect growth differences due to altered sex steroids production.

Key words: *bull, cortisol, flight speed test, IGF-I, steer.*

INTRODUCTION

In some production systems, growing calves are castrated to ease handling as well as to reduce sexual and aggressive behavior during the finishing phase. Castration alters growth rate and body composition in cattle due to a change in hormonal status (Henricks *et al.* 1988). The description of underlying mechanisms mediating the hormonal status in growing bulls compared to castrated calves usually comes from American literature where anabolic implants are used (Lee *et al.* 1990; Hunt *et al.* 1991; Schoonmaker *et al.* 2002). Thereby, the interactions between endogenous and exogenous effects of estrogenic hormones do not allow a detailed comparison with some animal husbandry systems that ban the use of growth promotants during fattening (i.e. European Union).

Some studies have proposed to delay castration until puberty (8 to 13 months of age, depending on the breed) to maintain the growth performance advantages of intact males until puberty and to obtain the benefits of castration on meat quality characteristics thereafter (Knight *et al.* 1999). Early maturing cattle breeds, such as Holstein, may be castrated before puberty and therefore slaughtered at younger ages (less than 12 months old) without detrimental effects on meat marbling. However, production parameters may be impaired. Lipid deposition features in dairy breeds may differ from unselected feral breeds and/or late maturing beef breeds that can be slaughtered at older ages (around 20–24 months of age). In this regard, insulin-like growth factor-I (IGF-I) is considered a short-term indicator of the animal nutritional status (Ellenberger *et al.* 1989), as well as a mediator of the growth response to endogenous estrogens and androgens (Lee *et al.* 1990).

Cattle temperament is the behavioral response to stressful events, such as handling by humans. Flight speed has been suggested as a consistent measure of temperament (Burrow *et al.* 1988), and this trait has been related to growth performance in beef calves (Hoppe *et al.* 2010).

The aim of this study was to compare the growth performance, plasma testosterone and cortisol levels around castration at 10 months of age, as well as plasma IGF-I concentration and temperament in intact bulls and steers from a feral beef breed (Serrana de Teruel) from 10 to 21 months of age.

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MATERIAL AND METHODS

Animal management and experimental design

This experiment was conducted at the facilities of the CITA Research Institute at Zaragoza (41° 42′ N, 0° 49′ W, 216 m asl, Ebro Valley, Northeastern Spain). All procedures were approved by the in-house Ethics Committee for Animal Experiments at the CITA of Aragon. The care and use of animals were in accordance with the Spanish Policy for Animal Protection RD53/2013, which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

Fourteen male calves from Serrana de Teruel breed were raised together from weaning at 4 months of age to 10 months of age. These cattle belonged to a dark or tabby-coated unselected breed which is seldom used to handling and it is raised in mountain areas of the center of Spain. During this earlier part of the growing period, all calves were fed concentrate plus straw *ad libitum*. Afterwards, young bulls (284 ± 25 days of age and 366.5 ± 48.5 kg live weight (LW), mean ± standard deviation) were assigned at random to one of two treatments: surgically castrated (steers) or intact (bulls). Castration was carried out using local anesthesia and analgesia with xylazine (5 mL Rompún®; Bayer, Leverkusen, Germany) and ketamine (5 mL Imalgene®; Mérial, Lyon, France), respectively. The fattening period was identical in both groups and it was divided into three feeding phases (Table 1).

Productive measurements

Feed intake (concentrate and barley silage) was registered daily on a group basis. Feed dry matter (DM) was determined at 60°C in a forced-oven until constant weight. Individual LW was recorded at weekly intervals during the fattening period and average daily gains (ADG) were estimated on a monthly basis by linear regression of LW against time.

Blood sampling and analyses

Blood samples (5 mL) were collected at monthly intervals by tail vessel puncture into vacuum heparinized tubes. Samples were centrifuged at 2500 × *g* for 15 min at 4°C. Plasma aliquots were prepared and stored at −20°C until analysis.

Peripheral testosterone and cortisol were analyzed at 10 months (pre-castration) and 11 months of age (post-castration) whereas IGF-I concentration was analyzed on a monthly basis from 10 to 21 months old. All hormones were analyzed through solid-phase enzyme-labelled chemiluminiscent immunometric assays (Immulite®, Siemens Medical Solutions Diagnostics Limited, Llanberis, Gwynedd, UK). In case of plasma IGF-I, bovine samples previously analyzed through a commercial enzyme immunoassay kit (Blanco *et al.* 2010) were re-assayed to evaluate the accuracy of the analysis. A regression analysis was performed to compare the results obtained with both methods (r = 0.96). The mean intra- and inter-assay coefficients of variance (CV) were 3.1% and 12.0%, respectively.

Flight speed test

A flight speed test of individual calves was determined by the method of Burrow *et al.* (1988) at 9, 10, 11, 12 and 20 months of age. This test imposed conditions of close human contact, social isolation and physical restraint on the calf. All animals were previously exposed to experimental conditions, which included a weighing scale, while undergoing common cattle management handling routines. Measurements were taken between 09.00 and 11.00 hours. Calves were moved as a group from their pen to the chute in a calm manner by the same experienced stock people each time, who were wearing working clothes. Immediately after the entry into the chute, the calves were identified from their ear tag and the order of entry to the chute was noted. Calves stayed in the weighing scale for 30 s until an unknown person for the calves opened the chute. The calves proceeded at their own pace along a single straight alley without seeing other calves. Flight time was the time, measured with a stopwatch by the same person, taken by a calf from the opening of the weighing scale until the two front extremities crossed 5 m. Flight speed was expressed in m/s.

Statistical analyses

Data were analyzed using the SAS statistical software (SAS Institute Inc., Cary, NC, USA). DM intake was analyzed with a general linear model (GLM procedure) considering gender and feeding phase as fixed effects. LW at the start of each feeding phase was analysed

Table 1 Feeding plan during the fattening period of steers and bulls from 10 to 21 months of age

Age, months	10–13	14–18	19–21
Phase	I	II	III
Months of the year	February–May	June–October	November–January
Feed	Concentrate† plus straw *ad libitum*	3 kg/day of concentrate plus barley silage‡ *ad libitum*	Concentrate plus straw *ad libitum*

†Concentrate contained 93.0% dry matter (DM), 11.7 kg MJ metabolizable energy (ME)/kg DM, 13.7% Crude protein (CP). ‡Barley silage contained 37.9% DM, 9.5 MJ ME/kg DM, 8.0% CP.

with a GLM procedure considering gender as a fixed effect and age at castration as a covariate. LW throughout the fattening period, ADG, plasma testosterone, cortisol and IGF-I levels were analyzed with repeated measures mixed linear model (MIXED procedure) considering gender and age period as fixed effects and the animal as a random effect. Data are reported as least square means and their associated standard errors. Multiple comparisons were performed by Tukey's method. The level of significance was set at 0.05, while tendencies were declared at levels between 0.05 and 0.10.

Spearman's rank correlation coefficients were used to evaluate the relationships between the order of entry into the chute when flight speed was measured, and to identify the consistency of temperament between months of test. In addition, Spearman's rank correlation coefficients were calculated for the association between order of entry and ADG, and flight speed and ADG at each age period.

RESULTS

Productive parameters

DM intake along the fattening period did not differ between steers and bulls (overall, 7.95 and 8.04 ± 0.45 kg DM/day, respectively; $P > 0.10$), but it was greater during the feeding phase I than during the feeding phase III on a metabolic LW basis (79, 75 and 64 ± 4 g DM/kg $LW^{0.75}$/day, in phase I, II and III, respectively; $P < 0.001$). The data of feed intake for steers and bulls at each period (I, II and III) are reported in Table 2. Animal LW prior to castration (10 months of age) did not differ between groups (367 ± 17 kg, $P > 0.10$), as it did not differ at the end of feeding phase I (or start of feeding phase II) (14 months of age) (487 ± 17 kg, $P > 0.10$). However, LW at the end of feeding phase II (or start of feeding phase III) (19 months of age) was already lower in steers than in bulls (552 vs. 623 ± 17 kg, $P < 0.05$), as it did at slaughter (21 months of age) (648 vs. 743 ± 20 kg, $P < 0.01$). Therefore, the mean ADG from 10 to 21 months of age was lower in steers than in bulls (0.99 vs. 1.23 ± 0.05 kg/day, $P < 0.001$), mainly due to the differences in the month following castration and the last fattening month (Fig. 1).

Peripheral testosterone and cortisol around castration

Plasma testosterone did not differ between gender groups at 10 months of age but, as expected, it was nearly undetectable in steers compared to bulls at 11 months of age (post-castration) ($P < 0.001$) (Fig. 2). Plasma testosterone increased in intact bulls from 10 to 11 months of age ($P < 0.05$). Nevertheless, plasma cortisol did not differ between steers and bulls at 10 and 11 months of age ($P > 0.10$) (Fig. 2).

Peripheral IGF-I

Plasma IGF concentration did not differ between steers and bulls at the start of the fattening period (phases I and II) ($P > 0.10$), and only some tendencies for lower plasma IGF-I concentration in steers than in intact bulls was found at 12 and 16 months of age ($P < 0.07$) (Fig. 3). Nevertheless, the differences between genders were strengthened during the last feeding period (phase III). Thereby, the mean plasma IGF-I concentration during the fattening period became lower in steers than in bulls (139 vs. 192 ± 17 ng/mL, $P < 0.05$).

Flight speed test

There were no differences in flight speed tests between groups throughout the fattening period ($P < 0.05$) but there was a tendency for greater flight speed in steers than in bulls at 11 ($P = 0.07$) and 12 months of age ($P = 0.08$). However, flight speed results did not differ between groups at the end of the fattening period (20 months of age) ($P > 0.10$) (Fig. 4). The overall flight speed of calves decreased throughout the fattening period ($P < 0.05$). Accordingly, there was a negative correlation between speed values and age period both in steers ($r_s = -0.47$, $P < 0.01$) and bulls ($r_s = -0.70$, $P < 0.001$).

No significant correlations were observed between order of entry in the chute and speed values at each session, either in steers ($P = 0.28$) or in bulls ($P = 0.80$). Similarly, no significant correlations was observed between order of entry in the chute and age period, either in steers ($P = 0.22$) or in bulls ($P = 0.09$). There was no correlation between order of entry and ADG, either in steers ($P = 0.92$) or in bulls ($P = 0.67$). In the same sense, there was no correlation between flight speed values

Table 2 Dry matter (DM) intake in steers and bulls at each feeding period from 10 to 21 months of age

	Feed intake (kg DM/day)		Standard error	Metabolic feed intake (g DM/ kg $LW^{0.75}$/day)		Standard error
Phase†	Steers	Bulls		Steers	Bulls	
I	7.57	7.52	0.83	81	78	7
II	8.27	8.43	0.65	76	74	6
III	10.71	10.85	0.83	88	84	7

†I = concentrate feed *ad libitum* (10–13 months of age), II = barley silage *ad libitum* and 3 kg of concentrate supplement (14–18 months of age), III = concentrate feed *ad libitum* (19–21 months of age). LW, live weight.

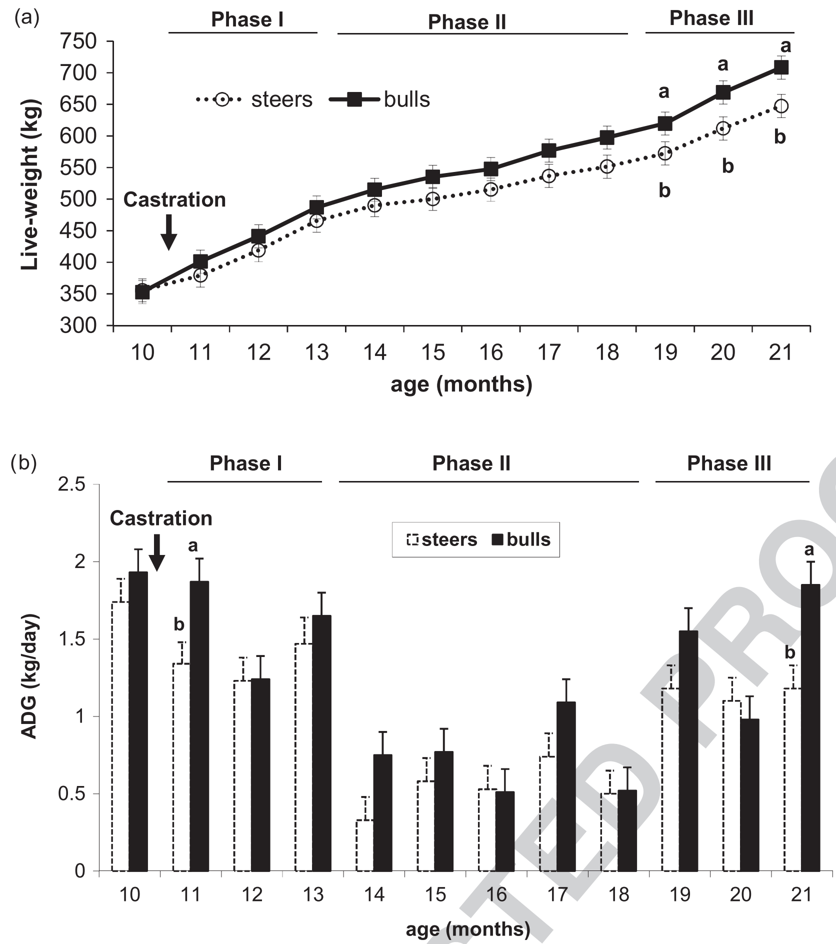

Figure 1 Live weight evolution (A) and monthly average daily gains (ADG) (B) in steers and bulls from 10 to 21 months of age (least square means ± standard error).

and ADG in steers ($P = 0.64$) and only a slight association was found in bulls ($r_s = 0.35$, $P = 0.07$).

DISCUSSION

Generally, it is assumed that steers grow slowly and deposit more fat than bulls (Lee *et al.* 1990). However, this assumption may be conditioned by the age at castration. The lower ADG in steers compared to intact bulls, especially during the month after castration, is in line with the results observed by Marti *et al.* (2010, 2013) in Holstein calves castrated at 3 or 8 months old and fed a similar concentrate-based diet. However, the ADG reduction was less marked when steers had been castrated at 5.5 months old (Pang *et al.* 2006), 7–9 months old (Cohen *et al.* 1990) or 13 months old (Ting *et al.* 2003) but fed forage-based diets (either good quality hay or grass silage supplemented with concentrate). Therefore, the difference in productive parameters between steers and bulls may be attenuated by forage compared to concentrate-based dietary strategies. Assuming that feed intake is controlled by physiological mechanisms and thereby it is linked to energy requirements (Decruyenaere *et al.* 2009), forage diets could be applied more efficiently during the earlier fattening period (10–18 months of age) since herein feed intake on a metabolic basis was greater in phases I and II than in phase III, even though during the last feeding period the animals were fed a concentrate-based diet.

In another close Spanish beef genotype (Parda de Montaña) castrated at 7–8 months of age and slaughtered at 18–19 months old (500 kg LW), the reduction in the productive performance of steers was modulated by supplementing with concentrate during the intermediate phase of the fattening period (concomitantly to the reduced forage availability season) instead of feeding a purely forage-based diet (Blanco *et al.* 2012), or by increasing the feeding level during the last fattening months (Blanco *et al.* 2014). In this study, *ad libitum* concentrate feeding in the last feeding period (phase III) did not counterbalance the lower ADG in steers compared to bulls.

In the current experiment, the ADG of intact bulls was kept within the range for other Spanish hardy genotypes (i.e. Asturiana de los Valles, Avileña) (1.30–1.34 kg/day) reared until 15 months of age

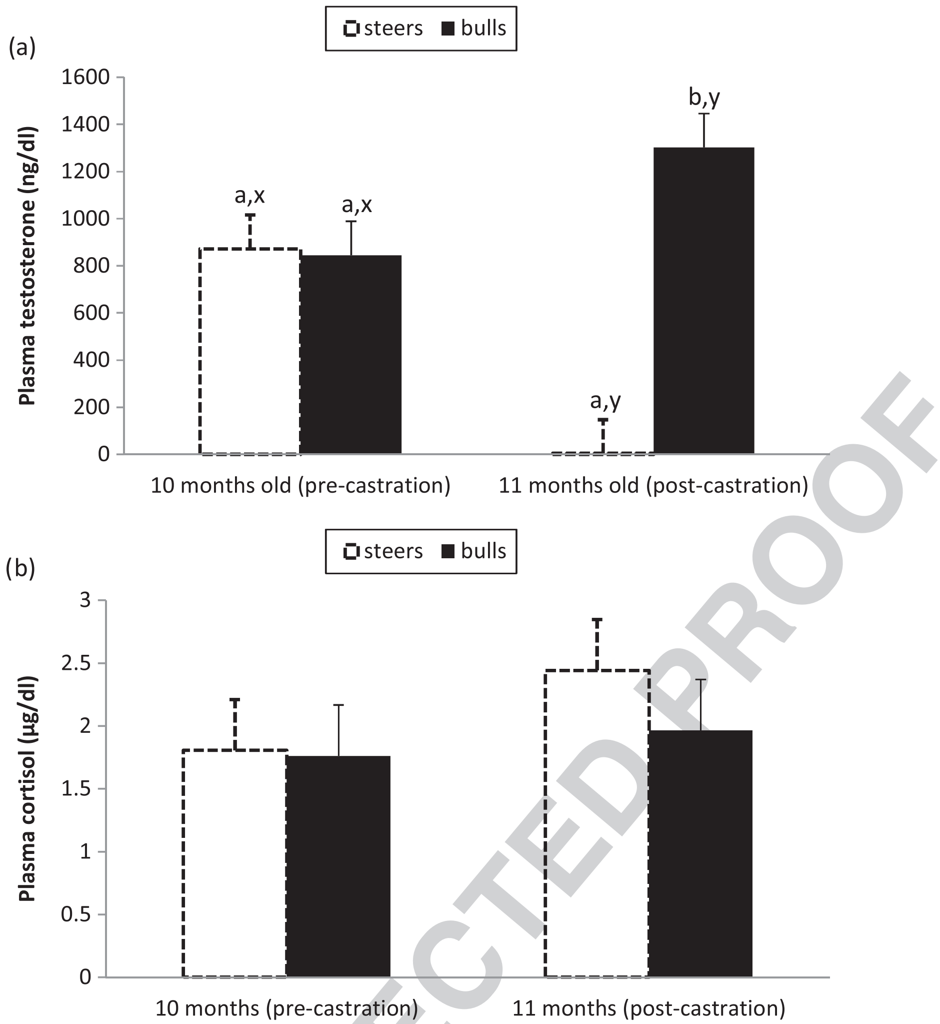

Figure 2 Circulating testosterone (A) and cortisol (B) in steers and bulls at 10 months (pre-castration) and 11 months of age (post-castration) (least square means ± standard error). [a,b]Denote statistical differences (at least $P < 0.05$) between gender groups within each age period. [x,y]Denote statistical differences (at least $P < 0.05$) between age periods within each gender group.

(550–560 kg LW) and fed a concentrate-based diet (Albertí *et al.* 2008). The present intact bulls reached a final LW of approximately 700 kg, even though they were previously fed a forage-based diet during 5 months (from 14 to 18 months old). Considering that mature male LW in this breed is around 840 kg (FAO 2010), the recorded bull LW during the last feeding period (phase III) (19–21 months of age) represented around 80–85% of their mature LW. Meat quality parameters from these animals are reported elsewhere (Ripoll *et al.* 2016). Briefly, the bulls showed lower fatness degree but greater conformation score and dressing percentage than steers. Accordingly, the intramuscular fat content was lower, whereas the edible meat content was greater in bulls than in steers. The age at castration may have played a role in the observed body growth and fat deposition pattern. Although delayed castration (9 to 12 months old) has been suggested in late maturing beef breeds to allow intact bulls growing at greater rates than steers until puberty (Knight *et al.* 1999), this practice is conditioned by the interval to the target slaughter age/weight, which may not be increased due to excessive feeding costs. Bretschneider (2005) reviewed this issue and concluded that surgical castration performed after puberty has an important detrimental effect on growth performance, which extends for a period beyond the first 30 days post-castration. The hypothalamic–pituitary-adrenal axis is activated by surgical castration and triggers a short-term acute increase (3-6 h) in plasma cortisol concentration that rapidly declines the day after castration (Cohen *et al.* 1990; Stafford *et al.* 2002). In this study, we measured only basal plasma cortisol the previous day (10 months of age) and four weeks after castration (at 11 months of age). At this moment, no differences between groups were observed, indicating that the release of this adrenal corticosteroid returned to baseline and chronic stress was no longer present.

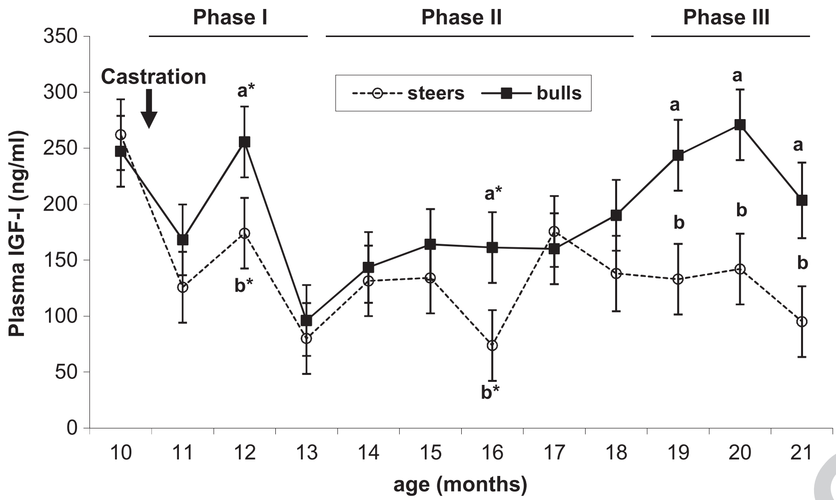

Figure 3 Plasma insulin-like growth factor-I (IGF-I) concentration in steers and bulls from 10 to 21 months of age (least square means ± standard error). Different letters ([a,b]) denote statistical differences (at least $P < 0.05$) between gender groups at monthly age periods. Different letters with asterisks ([a*,b*]) denote a tendency for statistical differences ($P < 0.07$) between gender groups at monthly age periods.

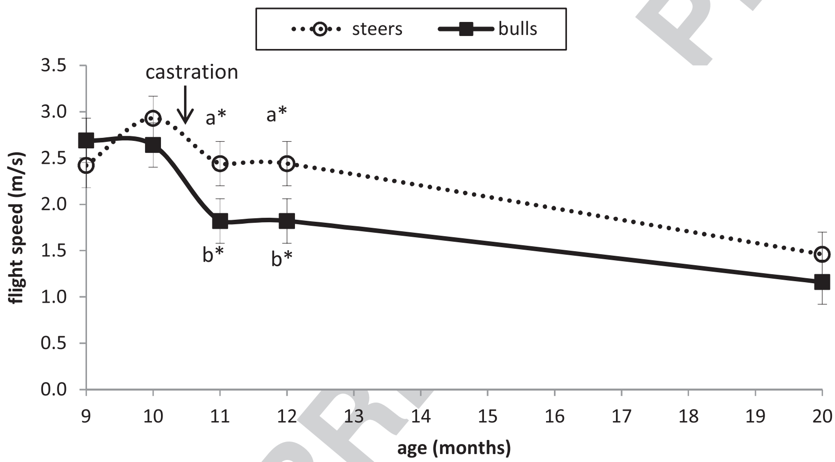

Figure 4 Flight speed test results in steers and bulls at 9, 10, 11, 12 and 20 months of age (least square means ± standard error). Different letters with asterisks ([a*,b*]) denote a tendency for statistical differences ($P < 0.08$) between gender groups at monthly age periods.

According to Govoni *et al.* (2003), IGF-I hormone plays an important role in regulating metabolic processes in skeletal muscle. However, the function and effect of IGF-I depends on the abundance of IGF-binding proteins (IGFBPs), which are responsible for regulating the amount of free IGF-I that is available to its receptors in the tissues, as well as transporting IGF-I in the bloodstream. In intensively managed young bulls slaughtered at 11 months of age, serum IGF-I concentration increased until it reached a plateau at 7–8 months of age which was parallel to maximal concentrate intake (Blanco *et al.* 2010). In other works comparing older intact bulls and steers, a gradual increase in IGF-I level was observed as sex steroids (i.e. testosterone) increased (mainly from 11 months of age onwards) (Lee *et al.* 1990) and concomitantly the testicles grew (Schoonmaker *et al.* 2002). Nevertheless, the afore-mentioned studies were carried out following animal husbandry procedures that involved the use of anabolic steroids both in steers and intact bulls. Collectively, in the previous results serum IGF-I concentration may be increased by gonadal hormones and thus the fact that the steer had been deprived of its endogenous androgen causes a decrease in circulating IGF-I, which is a hormone supporting growth (Lee *et al.* 1991). Whether an earlier age at castration may have equally modified the endocrine IGF-I pattern in these steers compared to bulls cannot be extrapolated. In any case, plasma level of IGF-I was highly correlated with empty body gain and protein deposition in late-maturing steers (Hayden *et al.* 1993).

In this study, plasma testosterone increased in intact bulls from 10 to 11 months of age, but this slight increase was not yet clearly reflected in greater circulating IGF-I, and the main differences in plasma IGF-I between steers and bulls appeared at 19 months of age. We hypothesize that the sustained rise in plasma IGF-I concentration in bulls but not in steers during the last feeding period (from 19 to 21 months of age) might be related to attainment of sexual behavior, suggesting that differences in peripheral IGF-I are due to gonadal rather than nutritional status. Indeed, Vijil *et al.* (2009) reported that the average age at ejaculate collection for appropriate preparation of semen doses in this breed was around 18 months of age.

Flight speed test may be useful to evaluate the fear response of the calf to being handled by humans (Müller and von Keyserlingk 2006). In this study, differences were observed in flight speed tests between groups, with a tendency for greater speed values in steers than in intact bulls 2 months after castration (11 and 12 months of age) but not at the end of the fattening period (20 months of age). Steers are more temperamental than bulls, even though total mounts per animal are reduced due to castration (Vanderwert *et al.* 1985). Compared to bulls, steers tend to have higher flight speeds and higher movement in the chute (Bruno 2015). Within each group, flight speed in steers was not associated with their monthly ADG, while flight speed values in bulls were positively associated with growth performance. This is not in agreement with the earlier suggested negative correlation between temperament scores and ADG, although this inverse relationship was deeper in some breeds (Brahman, Hereford, Limousin) than in others (Angus) (Hoppe *et al.* 2010; Cafe *et al.* 2011). Therefore, an additional study involving a greater sample size would be required to evaluate in this breed whether less docile animals are more or less productive.

If no septic lesions are found after surgical castration and the calves are kept clean by replenishing their straw bedding daily, behavioral evidence for chronic pain after surgical castration may be limited to small increases in the time spent standing abnormally over the first 2 weeks and small inconsistent increases in lesion licking the month after surgery (Molony *et al.* 1995). However, Devant *et al.* (2012) concluded that castration has a lasting reduction on physical activity because young bulls were more active (steps/hour) than steers (castrated at 3 months of age and slaughtered at 10 months).

In this study, even though the flight speed of steers and intact bulls was reduced with the course of the fattening period, the Serrana de Teruel cattle may be classified as a nervous breed when compared to values obtained by Fell *et al.* (1999), who classified Hereford and Hereford × Angus calves according to their flight speed values as nervous (1.9 to 2.8 m/s) or calm calves (0.6 to 1.4 m/s); or by Blanco *et al.* (2009), who classified as calm the progeny from Parda de Montaña (0.5 to 0.8 m/s) and even Pirenaica breeds (0.9 to 1.2 m/s). In fact, habituation to human handling may be more important than gender to determine cattle temperament. However, in this experiment there was no association between order of entry in the chute and speed values, or between order of entry in the chute and age at test, which confirms the individual variability of the behavioral response in this cattle breed as a possible consequence of their lacking genetic selection.

In conclusion, steers reared until heavy live-weights (21 months of age) grew slowly and had lower plasma IGF-I concentrations than their intact bull counterparts. These differences were mainly highlighted the month after surgery (11 months of age) and the last part of the fattening period (from 19 to 21 months of age). The present results confirm the role of IGF-I as a key anabolic hormone in male beef cattle and thus the measurement of this hormone may reflect growth differences due to altered sex steroids production. After surgical castration (11 and 12 months of age), steers showed a tendency to display greater flight speed values than intact bulls but baseline plasma cortisol concentration did not differ between groups at this time. At the end of the study, steers and bulls reached nearly similar temperament, as flight speed did not differ between them.

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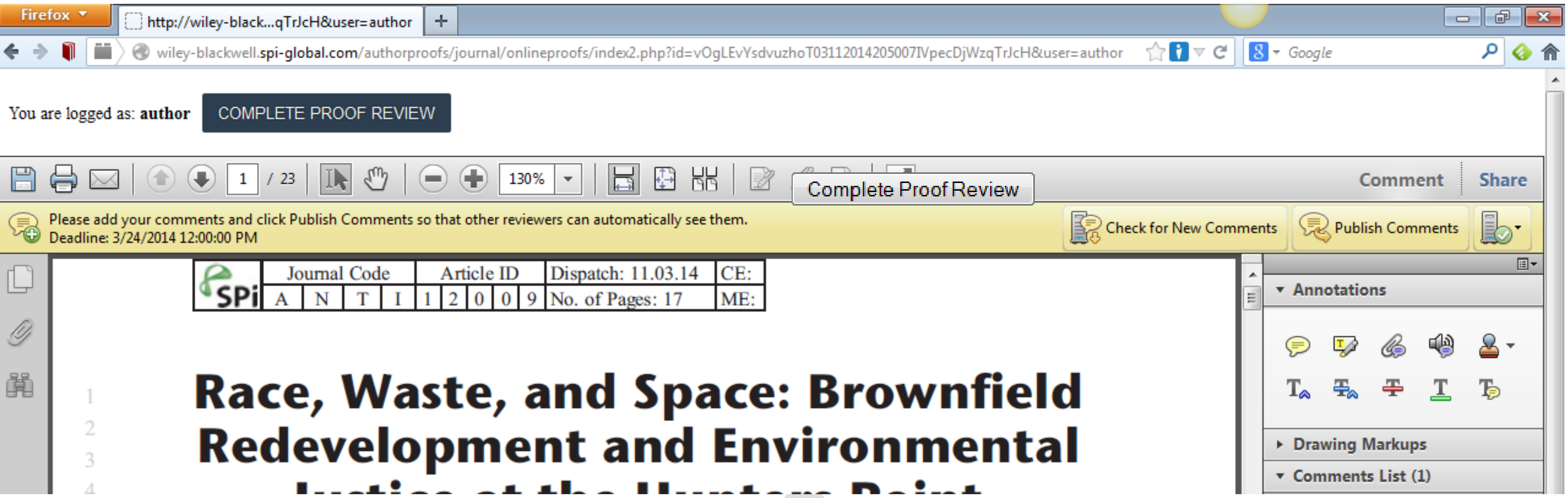

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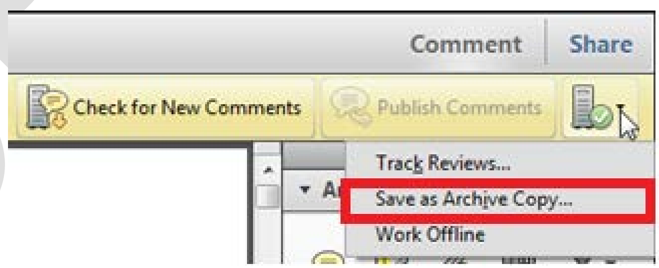

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