Livestock Science 140 (2011) 171–176

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Castration methods do not affect weight gain and have diverse impacts on the welfare of water buffalo males

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ARTICLE INFO

Article history:
Received 28 September 2010
Received in revised form 11 March 2011
Accepted 15 March 2011

Keywords:
Bubalus bubalis
Orchiectomy
Burdizzo
Chemical castration
Cortisol
Fibrinogen

ABSTRACT

Castration is used to improve the management of water buffalo beef males raised under extensive conditions. However, as buffalo are considered robust animals, their welfare is often neglected, which, among other implications, may compromise their productivity. The aim of this study was to determine the effects of different castration methods on the stress level and weight gain of water buffalo males. Two experiments were performed with three treatments each. In experiment 1, serum cortisol concentrations were used as stress indicators for non-castrated (control group) or castrated males, either surgically or by burdizzo clamp. In experiment 2, blood levels of fibrinogen were used as stress indicators for males in the control group compared to those castrated by either burdizzo clamp or intratesticular injection of calcium chloride (chemical castration). In both experiments, clinical parameters and the mean daily weight gain were measured for all males. Surgical castration and chemical castration caused higher stress than castration with burdizzo, with no differences observed in weight gain among castration methods. In conclusion, for water buffalo males, castration with burdizzo clamp is preferable to surgical and chemical methods because it causes the lowest stress level in the animals.

1. Introduction

Despite their important contribution for beef production, water buffaloes (*Bubalus bubalis*) have not been studied as frequently as other species. Thus, information about hematologic parameters (Silva et al., 1992) and inflammation markers in animals that are healthy (Khan et al., 1994) or suffering from different clinical conditions (Khan et al., 1997; Zaki et al., 2008) are scarce. In bovines, such parameters are well described and used to determine the most appropriate castration method, considering animal performance and welfare (Earley and Crowe, 2002; Fisher et al., 1996; Molony et al., 1995; Pang et al., 2006, 2008, 2009; Stafford et al., 2002; Thüer et al., 2007; Ting et al., 2003a,b). Studies that investigated the influence of castration on bubaline performance have been limited to evaluate characteristics of beef production, indicating that castrated males present an increased proportion of total fat (Charles and Johnson, 1975; Rodrigues et al., 2004) and produce a higher yield in certain carcass cuts (Jorge et al., 2007; Rodrigues et al., 2003), which is profitable for the beef industry. Such findings indicate that it is necessary to establish a feasible and efficient castration method that can preserve the welfare of bubaline males raised for beef production without impairing their growth performance.

A potentially feasible alternative is the intratesticular injection of a necrotizing chemical agent, such as cadmium chloride, which has been reported to be effective for bubaline castration (Costa et al., 2002). Nevertheless, this chemical can

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1871-1413
doi:10.1016/j.livsci.2011.03.026

cause restrictions on the consumption of the meat produced by the animals in which it is used. Therefore, chemicals used for this purpose should follow biosecurity guidelines and provide painless castration. In bovines, chemical castration using lactic acid reduces cortisol release, in comparison with castration using a burdizzo, even with the pain response elicited after the intratesticular injection (Cohen et al., 1990). Chemical castration using calcium chloride has also been described for bovines (Koger, 1978). When used in intratesticular injections at different concentrations in caprine and canine males, calcium chloride did not alter various stress parameters (Jana and Samanta, 2007; Jana et al., 2005), showing its potential as a chemical castration agent. Considering the lack of information about the influence of different castration methods on the performance and welfare of water buffalo males, it is of interest to determine the most suitable castration method for this species. This study aimed to determine the effect of classical methods of castration (surgical and burdizzo crushing) and of intratesticular administration of calcium chloride (chemical castration) on stress indicators and weight gain of water buffalo males.

2. Material and methods

Two experiments were conducted, under field conditions, on a private farm, located in Lages, SC, Brazil (lat 27° 46' 07, 63" S; lon 50° 17' 39, 83" O). On this farm, all males were kept grazing native pasture with *ad libitum* access to water and mineral salt and routinely submitted to surgical castration with a knife without use of either anesthetic or pain relief agents. In both experiments, all animals (41 crossbreed Murrah × Jafarabadi) wore a numbered earring for individual identification and a dyed collar to identify the treatment received.

The first experiment was conducted from September 5th to December 5th 2006 and the second experiment was conducted from August 7th 2007 to February 8th 2008. The methodology implemented in this study was approved (process number 1.44/09) by the Ethics Committee in Animal Experimentation of the Agro-veterinary Research Center – Universidade do Estado de Santa Catarina.

2.1. Experimental design

2.1.1. Experiment 1

Five days before the experiment began (D −5), 21 bubaline males, ranging from 7 to 18 months old, were weighed. Each group presented a total weight of 1308 ± 2 kg. The animals were randomly allocated to three treatments (seven animals per treatment): non-castrated (control); surgical castration; and castration using burdizzo clamp.

The animals were castrated on D0, and the following data were collected: serum levels of cortisol (D0: 0, 3, 6, and 9 h); average daily weight gain (D1 to D90); body temperature (D1 and D2); expression of local pain (D0, D1 and D2); and histological architecture of testicles from animals in the surgical (D0) and burdizzo (D90) castration groups.

2.1.2. Experiment 2

Sixty days before the experiment began (D −60), 20 males were weighed, identified, and randomly allocated to three treatments: non-castrated (control) (n = 6); castrated using burdizzo clamp (n = 7); and chemically castrated using an intratesticular injection of calcium chloride (n = 7).

The animals were castrated on D0, and the following parameters were evaluated: complete blood count (CBC) and blood chemistry (serum levels of calcium, glucose, urea, total protein, plasmatic protein, fibrinogen and creatinine) at D −4, D0, D1, D3, D7, D14, and D21; body temperature and pain expression to scrotal palpation in D0, D1, and D3; average daily weight gain from D3 to D267; and histological architecture of testicles of all castrated males at D267.

2.2. Treatments

The non-castrated males were submitted to the same restraining methods and management conditions as those applied to the castrated males. The surgical and burdizzo castration procedures were carried out without using either tranquilizers or anesthetic agents in order to reproduce the common field practice. For the surgical castration, the open method was used, with a single incision to remove an ellipse-shaped piece of skin at bottom of the scrotum, followed by exposure of the testicles, ligation of blood vessels and severing of the testicular cords using a scalpel. The removed testes were processed for histological evaluation.

Castration using a burdizzo clamp was performed through a single crushing of each spermatic cord and the associated scrotal tissue by compression for 1 min. Special attention was given to ensure that the clamp crush lines did not overlap, to preserve the blood supply to the scrotal tissue. The testes were surgically removed at the end of the experimental period, for histological evaluation.

For chemical castration, each testicle received an intratesticular injection of a solution containing 30% (w/v) calcium chloride ($CaCl_2$ $2H_2O$) and 2% (w/v) lidocaine chlorhydrate in apyrogenic water (Milli-Q syntesis, Millipore, EUA) sterilized through filtration in a membrane of 0.22 μm diameter pores (Millex GV, Millipore, USA). In each testicle, the dose was approximately 1 mL/100 kg of body weight, corresponding to the volume needed to make the testes turgid. For the injection, the testes were held in a position that allowed the introduction of a needle (22 G × 1") from the proximal extremity to the medial portion of the testicle. The testes were also surgically removed at the end of the experimental period for histological evaluation.

2.3. Blood collection

The first blood samples were collected at the same time that the treatment procedures took place, by restraining the animals in lateral recumbency with supporting ropes on the limbs. Subsequent blood samples were collected with the animals standing in a single-file chute with head gate. All blood samples were kept refrigerated in thermal boxes and sent for processing within 1–2 h.

In experiment 1, blood samples were collected from the jugular vein with a vacuum tube, without anticoagulant, for serum preparation. Serum samples were obtained from the blood samples on D0, at 0, 3, 6, and 9 h, split into different previously identified tubes and stored at −20 °C, until cortisol analysis.

In experiment 2, blood samples were collected from the jugular vein using a syringe with an 18 G × 1½" needle. The collected blood was immediately split into three tubes: without anticoagulant to obtain serum; with anticoagulant (EDTA) for CBC and fibrinogen determination; and with fluoride for glucose measurement.

2.3.1. Sample processing

In experiment 1, serum cortisol concentration was measured by solid phase radioimmunoassay using the Coat-A-Count® Cortisol kit (Siemens Medical Diagnostics, USA), according to the manufacturer's instructions. The analysis of sensitivity (minimum dose) was of 91% (0.04 ug/dL) and the intra-assay variation coefficient was between 2.56% (lowest) and 5.22% (highest).

In experiment 2, blood samples with EDTA were analyzed immediately after collection for CBC through electronic cell counting (ABC Vet, Horiba ABX, France). The differential leukocyte count and blood cells' morphologic characterization were obtained via blood smears (Jain, 1993).

The packed cell volume (PCV) was obtained by centrifugation. Subsequently, the hematimetric indices, the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC), were calculated (Jain, 1993).

The concentration of plasma protein was measured through refractometry and the fibrinogen dosage through heat precipitation and refractometry.

The serum concentration of calcium, glucose, urea, creatinine and total protein were determined by photometry (Spekol® UV VIS spectrophotometer, Carl Zeiss, Germany) using Calcium Liquiform, Glucose Pap, Urea UV Liquiform, Creatinine, and Total Proteins kits, following the manufacturer's recommendations (Labtest Diagnóstica S.A., Brazil).

2.4. Assessment of body temperature and pain expression

The animals were clinically evaluated to assess body temperature and behavioral response during the castration procedure. Immediate expression of pain during castration was evaluated using the following score: 0, no response; 1, mild struggling; 2, struggling with hind and front limbs; and 3, massive struggling that involved the whole body (Thüer et al., 2007). At 24 (D1), 48 (D2), and 72 h (D3), body temperature and scrotal pain response was evaluated by manual palpation in the standing position, and classified according to the following score: 0, no response; 1, mild foot stamping under manipulation; and 2, strong foot stamping under simple touch.

2.5. Assessment of behavior

In experiment 2, during the first hour after castration, all males were kept in the management stalls for evaluation of their behavior. Afterwards, the animals were released into a pasture, where their behavior was observed for an additional 5 h (Molony et al., 1995; Thüer et al., 2007).

2.6. Recording of body weight

The males were individually weighed at D −5, D1, D2, D30, D60, and D90, in experiment 1, and at D −60, D3, D30, D60, D90, D131 and D267 in experiment 2. The weighing was always done during the morning, after withdrawing the animals from the native pasture.

2.7. Histological examination of the testes

After surgical removal, the testis were sliced longitudinally and fixed in formalin 10% (v/v). A section of 5 μm thick was cut from the middle portion of each formalin-fixed testis, stained with hematoxylin–eosin (HE) and examined under light microscopy at 100× and 400× magnifications. The structure of the seminiferous tubules and interstitial spaces were examined.

2.8. Statistical analysis

The data were submitted to analysis of variance and the means were compared using Tukey test (MINITAB, State College, EUA). Differences were considered significant when $P<0.05$.

3. Results and discussion

This study focused on determining the effect of different castration methods on the welfare of water buffalo males. In experiment 1, only the most commonly known methods were performed, whereas in the second experiment chemical castration with calcium chloride was used for the first time in bubaline castration. In experiment 1, during castration, the males submitted to surgical castration showed intense pain expression (score 3) in five situations: during incision of the scrotal skin; during the opening of both the tunica vaginalis and the tunica albuginea; and during the cut of each spermatic cord. The animals castrated with a burdizzo clamp showed intense pain expression (score 3) during the crushing of each spermatic cord. Thus, castration using burdizzo clamp appeared to be less traumatic than the traditional surgical castration. During the first 24 h post-treatment (D1), all males lost weight, evidencing the effect of food restriction and intense management during successive blood drawings during a 9-h interval on D0. By D2, males castrated with burdizzo had not recovered their previous body weight, whereas those that were surgically castrated and those in the control group recovered their body weight at levels similar to those previously observed at D5. These results suggest stress in the males castrated with burdizzo at that period, but at the time of the last weighing (at D90), there was no difference ($P>0.05$) in the average daily weight gain (ADG) between non-castrated males (0.312 kg/day) and those castrated either surgically or using a burdizzo clamp (0.396 and 0.349 kg/day, respectively). These results are similar to those previously described for bubalines (Bento et al., 2000) and bovines (Bretschneider, 2005; Stafford et al., 2002).

The body temperature of non-castrated and castrated males ranged from 38.9 to 39.9 °C during the clinical assessments, with no signs of systemic disorders. The castrated animals expressed signs of moderate pain on palpation (Score

1), indicated by flexed hind limbs and arched back, followed by mild foot stamping. This behavior was less characteristic than that reported for bulls castrated with burdizzo (Thüer et al., 2007). At D2 surgically castrated males still expressed remarkable pain on palpation (Score 2), whereas those castrated with burdizzo, despite presenting swelling of the scrotum at D1 (Fig. 1, B2), expressed sensitivity similar to that observed in non-castrated males (Score 0). Scrotum distension probably caused the discomfort which resulted in delayed weight recovery.

At the time of the first blood sampling (0 h of D0), the baseline serum cortisol level was assessed for all males (0.35 to 0.38 μg/dL). At the 3rd h of D0, the serum cortisol concentration for non-castrated males (0.63 μg/dL) and for those castrated with burdizzo (0.83 μg/dL) were both lower ($P<0.05$) than those observed for surgically castrated males (1.58 μg/dL). At the 6rd h of D0, the serum cortisol concentration was similar to that observed at the 3rd h of D0 (0.51 μg/dL for non-castrated males, 1.35 μg/dL for surgically castrated males and 0.80 μg/dL for males castrated with burdizzo). This stabilization of serum cortisol levels was also observed for surgically castrated bulls (Fisher et al., 1996). Nevertheless, such results contrast with the report of occurrence of maximum serum cortisol concentration 6 h after surgical castration in bulls (Cohen et al., 1990). At the 9rd h of D0, serum cortisol levels in non-castrated males were 0.67 μg/dL, but dropped to 0.56 μg/dL in the surgically castrated males. Such a decrease also contrasts with studies that reported increased serum cortisol levels for up to 12 h after castration, in bulls (Earley and Crowe, 2002; Ting et al., 2003a). At the 9rd h of D0, males castrated with burdizzo presented high serum cortisol concentration (0.98 μg/dL), tending to express chronic pain. Such a cortisol concentration diverges from those measured for bulls castrated with burdizzo, which started to decrease at 1.5 h after castration (Stafford et al., 2002; Tüher et al., 2007).

During the experimental period, the animals castrated with burdizzo reduced testicular volume remarkably, but their testicle consistency was harder than that for non-castrated males. After the healing of the scrotal skin lesions, males castrated with burdizzo no longer showed signs of pain (Score 0).

The testicles collected from surgically castrated males at D0 were used as the standard testicular histological arrangement for the microscopic evaluation of all testes (Fig. 1, A1). The testicles collected from males castrated with burdizzo at D90 presented massive degeneration and necrosis, macrophage infiltration and mineralization of the seminiferous tubules (Fig. 1, B1), which characterizes a severe degeneration.

In experiment 2, the immediate pain expression during burdizzo crushing was similar to that described for males castrated with burdizzo in experiment 1. For chemical castration, while the needle was introduced through the scrotal skin no pain expression was observed; the animals reacted slightly, only expressing mild struggling (Score 1). That mild pain expression is highly desirable during castration procedures, indicating that chemical castration can be less traumatic than castration with burdizzo clamp. After castration and blood sampling, non-castrated and castrated males were monitored inside a holding pen for 1 h. No evidence of change in behavior was observed for the males in

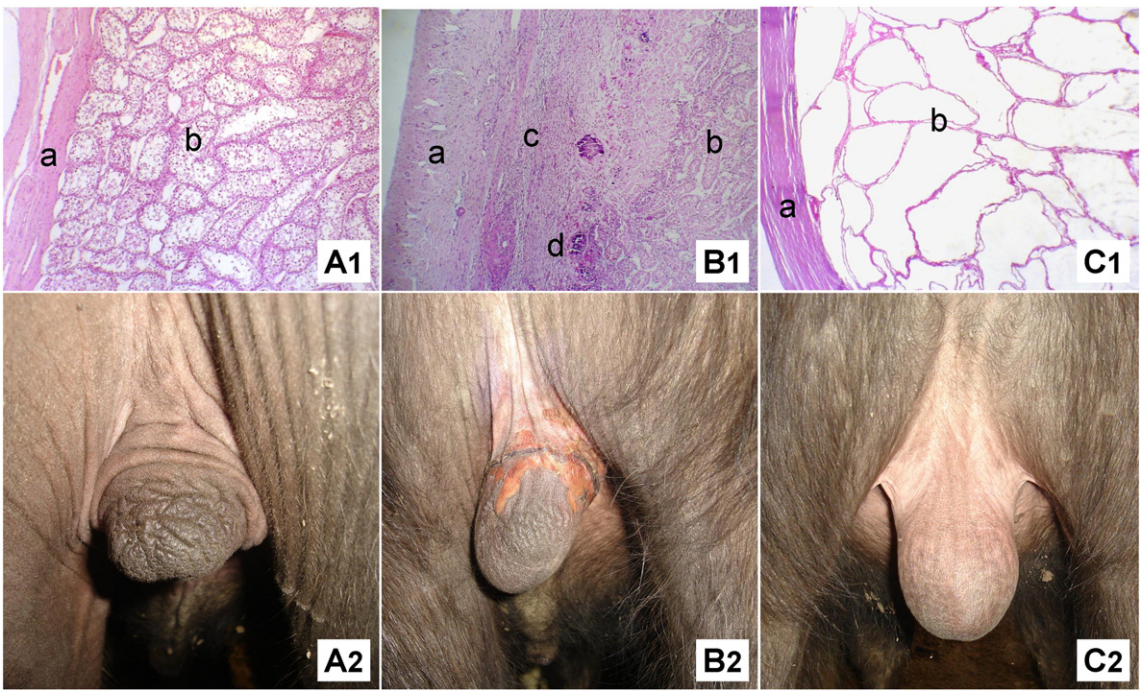

Fig. 1. A = Control; A1: normal tunica albuginea (a) and seminiferous tubules (b) from surgical castrated animal at D0; A2: scrotum from a non-castrated male; B = Burdizzo; B1: normal tunica albuginea (a), degeneration and massive necrosis of the seminiferous tubules (b), macrophage infiltration (c) and mineralization of seminiferous tubules (d) at D267; B2: swollen scrotum, with evident burdizzo crushing lines; C = Chemical castration; C1: normal tunica albuginea (a) and disappearance of epithelial cells, with remaining basal membrane (b) at D267; C2: swollen scrotum at D3. *A1, B1 and C1 were stained with HE.

any group; they remained ruminating in normal standing or lying posture, denoting a low stress level situation.

After being transferred to the pasture, males from all treatments stayed moving around, showing short periods of grazing, diverging from the behavior usually expressed by bulls, which remain motionless after either surgical castration or castration with burdizzo (Molony et al., 1995). During relocation, various males showed changed paces, characterized by difficulty in extending the hind limbs. As the non-castrated animals were submitted to the same method for limb restraint and they also showed this behavior, it seems that the method of restraint is responsible for this finding. During the clinical examination conducted along with the blood sampling at D1 and D3, non-castrated males and those castrated with burdizzo presented mean body temperature of 38.0 °C (37.2 to 38.8 °C), whereas the body temperature for chemically castrated males was slightly higher , averaging 38.7 °C (37.8 to 39.6 °C). An increase in scrotal size and pain expression on palpation was observed for males castrated with burdizzo, as also observed in experiment 1 for the same treatment. Chemically castrated males presented an evident increase in the scrotal volume (Fig. 1, C2), characterized by edematous scrotal skin and swollen testicles. Even though they presented a distended scrotum, these males expressed no pain (Score 0) response at palpation, showing lower response than that observed for non-castrated animals (Score 1). In the chemically castrated males the local inflammation is attributed to free radical release caused by the calcium reaction (Jana and Samanta, 2007; Jana et al., 2005).

In this study, no difference was observed ($P>0.05$) in hematologic parameters (RBC, leukocyte count and differential, bands, neutrophils, lymphocytes, eosinophils, basophils, and monocytes, HGB, PCV, MCV, and MCHC values) among treatments (Table 1), which contrasts with findings observed for bulls (Ting et al., 2003a).

Table 1
Mean (±SD) for hematologic parameters from blood samples collected from D0 to D14 for non-castrated males and for males submitted to castration with burdizzo and chemical castration.

Parameter	Non-castrated	Burdizzo castration	Chemical castration
RBCs ($\times10^6$/μL)	10.2 ± 2.1	10.2 ± 1.7	10.3 ± 1.8
HGB (g/dL)	13.2 ± 2.9	13.1 ± 2.4	13.3 ± 2.5
PCV (%)	40.0 ± 8.7	39.9 ± 7.0	40.4 ± 7.4
MCV (/fL)	39.5 ± 8.4	39.3 ± 6.6	39.5 ± 7.1
MCHC (g/dL)	33.0 ± 6.8	32.9 ± 5.3	33.0 ± 5.7
Leucocytes (/mL)	11556.1 ± 3.0	11273.5 ± 2.9	10860.7 ± 3.0
Bands (/mL)	49.4 ± 4.1	57.7 ± 3.9	38.5 ± 4.1
Neutrophils (/mL)	3128.8 ± 12.2	3215.2 ± 11.5	2894.3 ± 12.3
Lymphocytes (/mL)	7560.6 ± 11.9	7037.5 ± 11.2	7067.7 ± 12.1
Eosinophils(/mL)	75.7 ± 9.7	222.4 ± 9.1	233.3 ± 9.9
Basophils (/mL)	202.2 ± 1.9	412.3 ± 1.8	262.2 ± 1.9
Monocytes (/mL)	684.6 ± 211	648.2 ± 211	578.1 ± 208
Calcium (mg/dL)	11.7 ± 3.5	11.4 ± 0.9	11.3 ± 1.2
Glucose (mg/dL)	75.8 ± 14.1	79.7 ± 19.5	72.8 ± 9.9
Urea (mg/dL)	61.3 ± 15.5	57.7 ± 15.9	60.2 ± 15.0
Total protein (mg/dL)	6.8 ± 1.2	6.6 ± 0.7	6.6 ± 0.6
Plasmatic protein (g/dL)	6.4 ± 1.3	6.5 ± 1.1	6.6 ± 1.1
Fibrinogen (mg/dL)	647.1 ± 187[a]	682.1 ± 175[b]	692.2 ± 179[c]
Creatinine (mg/dL)	0.3 ± 0.3	0.2 ± 0.2	0.2 ± 0.2

[a,b,c] Means having distinct superscripts in the same line differ by $P<0.05$.

The RBC count and the HGB, PCV and MCV values were similar to those obtained from healthy animals within the same age range for the Jafarabadi and Murrah breeds (Silva et al., 1992). No changes were observed ($P>0.05$) in the levels of calcium, glucose, urea, creatinine and both total and plasmatic protein, as also described for bulls (Cohen et al., 1990). The only change detected in hematologic parameters among treatments was the increase in fibrinogen concentrations in the samples from both castration treatments (Table 1), as also reported for bulls (Earley and Crowe, 2002; Ting et al., 2003a). The increase in fibrinogen levels is characterized by an increase in hepatic production in response to interleukins 1 and 6 and the tumor necrosis factors generated during inflammatory or traumatic conditions (Jain, 1993; Meredyth and Robin, 2007). This fibrinogen response allows its concentration to be used as an indicator of acute or chronic inflammation in ruminants (Khan et al., 1994, 1997). In our study, as the highest fibrinogen plasma concentrations ($P<0.05$) were observed for chemically castrated males, with the highest measurement occurring at D14 (data not shown), it could be inferred that chemical castration induces greater stress than castration with a burdizzo clamp.

As also observed in experiment 1 (D90), the testicles from males castrated with burdizzo presented a marked reduction in volume and an increased consistency at palpation, at the end of experiment 2 (D267). Nevertheless, two of the animals presented one testicle with similar characteristics to those of the non-castrated males, which supports the assumption that castration using the burdizzo clamp may result in occasional failures (Stafford et al., 2002). Such failures may be attributed to the operator, if the spermatic cord is not properly held into the burdizzo clamp when it is being closed. In young water buffalo males, the presence of large skin folds and a scrotal neck that is not clearly evident (as in Fig. 1, A2) reinforces the need of operator's care to prevent failures on burdizzo castration. No failures were observed among chemically castrated males; the testicles were reduced in size and presented harder consistency, which was followed by a decrease in mobility due to adherence to the adjacent structures. In the histological evaluation, the testicles from the animals castrated with burdizzo showed changes similar to those observed in experiment 1 (Fig. 1, B1), with the exception of the two testicles that failed to be emasculated. The testicles of chemically castrated males were depleted of epithelial cells, containing only basal membranes (Fig. 1, C1). Although such alterations characterize extensive degeneration with low possibility for regeneration, the degree of degeneration caused by the calcium chloride in the bubaline testicles was not so intense as that observed for testicles of bulls (Koger, 1978), bucks and dogs (Jana and Samanta, 2007; Jana et al., 2005). That would occur because the intensity of such a response is directly dependent on the calcium chloride concentration in the injected solution (Jana et al., 2005). Thus, in our study, the use of a 30% (w/v) calcium chloride solution at 1 mL per 100 kg of body weight was not enough to produce a complete testicular fibrosis.

At the last weighing, at D267, no difference in growth performance was detected among treatments ($P>0.05$). Non-castrated males gained 0.375 kg/day, whereas males submitted to castration with burdizzo or chemical castration gained 0.436 and 0.339 kg/day, respectively. These results are similar

to those observed in experiment 1 and comparable to those from Bento et al. (2000). As such animals are commonly raised for beef production, an increase in total fat percentage (Charles and Johnson, 1975; Rodrigues et al., 2004) and a higher yield in special meat pieces (Jorge et al., 2007; Rodrigues et al., 2003) may be desirable. However, the lack of difference in the average daily weight gain among the castrated and non-castrated animals may justify eliminating the practice of castrating water buffalo males, in order to preserve their welfare.

4. Conclusions

Castration with burdizzo clamp caused less change in the serum cortisol levels than surgical castration and induced a lower amount of acute phase protein (fibrinogen) than chemical castration with calcium chloride. Therefore, castration of water buffalo males with a burdizzo clamp induces less stress than other castration methods and is the least detrimental method considering the animal's welfare.

Acknowledgements

The authors thank Mr. Antônio Cesar Martins for providing the animals used in the implementation of this study and Mr. Wagner Marques de Lima and Mr. Casemiro Marin for the financial support. The authors are also thankful to Dr. Thomaz Lucia Junior for the careful reading of the manuscript.

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