Effects of castration and weaning conducted concurrently or consecutively on behaviour, blood traits and performance in beef calves

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The objectives of this study were to evaluate the effects of Burdizzo castration and abrupt weaning on the behaviour, blood traits and performance of beef calves when weaning was conducted concurrently or consecutively to castration. In total, 64 male beef calves aged between 6 and 7 months were assigned to a 2 × 2 factorial design with the following treatment groups (n = 16 animals per treatment): (1) castrated and concurrently weaned in week 0 (CAS-WEA); (2) castrated in week 0 and weaned in week 4 (CAS-CON); (3) bulls weaned in week 0 (BUL-WEA); and (4) bulls weaned in week 4 (BUL-CON). The behaviour of the calves was observed for 3 days following weaning. Blood was collected weekly from weeks 0 to 5 and analysed for the acute-phase protein haptoglobin, and neutrophil and lymphocyte percentages. BW was recorded weekly from weeks 0 to 7. Animals were slaughtered at 17 months and weight, dressing percentage and carcass classification were recorded. On day 1 after weaning, the number of vocalizations (calls/10 min) was higher in BUL-WEA (7.2) and CAS-WEA (5.4) than in calves of CAS-CON (2.8) and BUL-CON (2.9) groups (P < 0.05). From days 1 to 3 vocalizations decreased in all groups. CAS-CON and BUL-CON animals spent 20% lying on day 1 after weaning compared with 40% in CAS-WEA and BUL-WEA calves (P < 0.05). The haptoglobin concentration decreased during the first 5 weeks after weaning in all groups independent of the castration, weaning group or its interaction (P > 0.05). WEA groups showed an increased average daily gain (ADG) during weeks 0 to 3 and a reduced ADG during 4 to 7 weeks in comparison with CON animals. At slaughter, bulls were about 80 kg heavier than castrates and had a superior dressing percentage and carcass classification (P > 0.05). In conclusion, weaning had a greater effect on the number of vocalizations, standing/walking and lying behaviour and ADG compared with Burdizzo castration. In comparison with undertaking the procedures separately, concurrent castration and weaning neither affected behaviour and haematological parameters nor impaired performance. There was no evidence that the concurrent application of both treatments markedly increased the stress response compared with their application at intervals of a few weeks.

Keywords: abrupt weaning, beef calves, behaviour, blood traits, burdizzo castration

Implications

In steer production systems, castration and weaning are the main stressors experienced by calves. In this study, the effect of weaning conducted concurrently or consecutively with castration by the Burdizzo method on the stress response and performance was evaluated. Calves that were castrated and weaned concurrently did not show an enhanced response to the management procedures. Compared with Burdizzo castration, weaning was found to be the greater stressor for the animals. This highlights that applying both treatments at the same time does not markedly increase the stress response compared with performing these procedures at intervals of a few weeks.

Introduction

In steer production systems, castration is, apart from weaning, the major management procedure causing distress in calves, which can lead to reduced feed intake, growth rates and even weight loss during the first weeks after the procedures are conducted (Arthington et al., 2003; Ting et al., 2005; Warnock et al., 2012).
Weaning is commonly practised by abrupt separation characterized by physical separation of mother and young and includes the immediate milk withdrawal for the calves. Calves respond to weaning with increased vocalizations, agitation, walking activity and reduced feed intake and ruminination (Veissier and Le Neindre, 1989; Haley et al., 2005) resulting in a suppressed immune system and enhanced disease susceptibility (Lefcourt and Elsasser, 1995).

An alternative weaning strategy is two-stage weaning with the calves being prevented from suckling the dam by fitting an anti-suckling device (nose-flap) before they are separated from the dam (Haley et al., 2005; Boland et al., 2008). Studies in lambs emphasized the suitability of this method to reduce weaning stress (Schichowski et al., 2008). As another alternative, fence-line weaning allows the calf to have a certain level of social contact to the dam for several days while being prevented from suckling (Stooley et al., 1997; Price et al., 2003). However, these methods have drawbacks, and are for several reasons not widely used under practical conditions to reduce weaning stress in beef calves.

Castration of the male calves causes a degree of pain and stress, whereas the animal’s response to it is probably dependent on several factors such as the age, method of castration, and use of anaesthetics and analgesics (Ting et al., 2003; Coetzee et al., 2008; Currah et al., 2009). The most commonly used physical castration methods can be divided into methods in which the testicles are removed surgically or the blood supply is eliminated (Stafford and Mellor, 2005). Cutting off the blood supply with elastic rubber rings is labour extensive and a safe method, though the animal well-being is impaired (Booker et al., 2007; Stafford, 2007). Therefore, this procedure is forbidden in some countries like Germany. Alternatively, it can be done by using a Burdizzo clamp, which application causes the crushing of the spermatic cords and associated blood vessels within the scrotum, leaving the scrotum intact without any wound. Castration by the Burdizzo method is frequently used in various countries (Stafford et al., 2000; Boesch et al., 2006; Coetzee et al., 2010). During the first days after castration, a swelling of the scrotum is reported independent of the age of the calves at castration (Molony et al., 1995; Ting et al., 2005; Pang et al., 2008).

For the assessment of the stress caused by either weaning or castration, vocalization is a useful indicator measuring the response of the calves to distress, signalling the physiological and emotional state, motivations and intentions of the calling animal (Watts and Stookey, 2000). Furthermore, changes in the behaviour of the calves can be observed following weaning (Veissier and Le Neindre, 1989; Haley et al., 2005) and castration (Molony et al., 1995) such as in increased agitation, walking activity and reduced feed intake and ruminination.

Changes in different blood traits can also be observed following stressful situations. These changes are expressed as an increase in leucocyte numbers and a decreased proportion of lymphocytes following weaning (Hickey et al., 2003; Lynch et al., 2010). After castration increased numbers of leucocytes are commonly found (Ting et al., 2003). Arthington et al. (2003) reported that acute-phase proteins, which are inflammatory mediators, increased after weaning and transportation.

The easiest way to reduce weaning stress is to minimize other management processes undertaken at the same time as weaning. However, studies evaluating the effects of weaning conducted concurrently or consecutively to castration on stress responses of beef calves are unknown. Therefore, the objective of this study was to evaluate the effect of castration on the stress response assessed by behavioural observations as well as blood traits and performance of calves weaned at the same time of castration or 4 weeks afterwards.

Material and methods

The study was conducted on the experimental farm of the Georg-August-University Göttingen, Germany. The farm is located in Lower Saxony at an altitude of 220 to 280 m above sea level with an average precipitation of 900 mm per year and an average annual temperature of 8.2°C.

Animals, treatments and management

Over 2 consecutive years, a total of 64 male beef calves (Limousin × Blonde d’Aquitaine and Aubrac × Blonde d’Aquitaine crossbreds) born in spring were examined in this study. At the end of the pasture season, when the calves were aged 6 to 7 months and weighed 253 kg (s.e. 6.0 kg) on average, the cow–calf pairs were moved from the pasture to the barn and kept in four straw-bedded compartments of equal sizes, thus each pen accommodated eight cow–calf pairs. After 1 week, which was defined as week 0, the 64 studied calves (n = 32 per year) were assigned to a 2 × 2 factorial design with the two factors castration and weaning: (1) Castrated and concurrently weaned in week 0 (CAS-WEA); (2) castrated in week 0 and weaned in week 4 (CAS-CON); (3) bulls weaned in week 0 (BUL-WEA); and (4) bulls weaned in week 4 (BUL-CON).

One week before castration, all animals of the suckler cow herd were moved from pasture into the barn. After weighing, the cow–calf pairs of the four different treatment groups were allocated to one of four straw-bedded compartments of equal size, thus each pen accommodated eight cow–calf pairs. At the day of castration, all calves were weighed and a blood sample was collected from each individual animal as described below. CAS-WEA and BUL-WEA calves were separated from their dams into two straw-bedded pens of equal sizes adjacent to each other (12.4 m²/calf) within auditory distance of their dams. Thus, the weaned calves and their dams were able to have vocal communication with each other. There was a feed bunk in front of the pen with 1.1 m²/calf. The animals of the castrated and intact bull groups were able to have visual, tactile and olfactory contact to each other.

CAS-CON and BUL-CON calves remained with their dams in their respective pens for another 4-week period. After this
period, weaning was done by separating the calves from their dams and placing them into the weaning barn. They were housed in the same way as described for the other two groups. After weaning of the CAS-CON and BUL-CON calves, all calves of the trial were kept in the weaning barn for a period of 3 weeks. After this 3-week period, all calves were regrouped within castration group, and moved into a further separate stable for the following fattening period.

As long as the calves were housed with their dams, they had access to the cows’ feed (straw and grass silage). The weaned animals were supplied with a feeding ration consisting of grass silage (9.9 MJ metabolizable energy/kg; 16.5% CP), but without any concentrates. One week before the fattening period started, the feeding ration of the calves was replaced stepwise by maize silage. During the fattening period, the animals were offered maize silage ad libitum and an additional amount of 2.5 kg concentrate per animal and day. The concentrate consisted of field bean (65%), barley (32%) and minerals (3%).

Castration and blood sampling
The Burdizzo castration of the calves was performed between 6 and 7 months of age in accordance to the German Animal Welfare law by an experienced veterinarian in week 0. The animal was restrained first in a crush and additional ropes were used to hold its hind legs in a spread position to prevent injuries to the veterinarian due to movements of the animal. Calves received an injection of xylazine (0.25 ml/100 kg BW) through the distal pole of each testicle. The castration was performed from behind through the legs of the calf. Each spermatric cord was crushed twice using the Burdizzo tool.

Blood was collected by jugular venipuncture weekly until 5 weeks following castration. The values measured in week 0 were used as pre-treatment baseline values, and therefore blood samples were taken before the treatment procedures (castration and weaning) were conducted. Similarly, in week 4 blood sampling was performed immediately before the weaning of the CAS-CON and BUL-CON animals. Unclotted whole blood samples collected into EDTA tubes were analysed for neutrophil and lymphocyte counts using a blood analyser (Cell-Dyn 3500CS; Abbott Laboratories, Abbott Park, IL, USA) at the Institute of Veterinary Medicine of the Georg-August-University Göttingen, Germany. Neutrophil and lymphocyte counts were used to calculate the neutrophil : lymphocyte ratio.

Blood samples for plasma haptoglobin determination were collected into heparinized tubes and centrifuged at 3000 r.p.m. at room temperature for 10 min at the laboratory of the Department of Animal Science, Georg-August-University Göttingen, Germany. Plasma was subsequently stored at −20°C until assayed for the acute-phase protein haptoglobin at the Institute of Animal Sciences, University of Bonn, Germany, as described by Orro et al. (2008).

Behavioural observations
Direct observations were performed to determine the calves’ behaviours using the scan sampling methodology. The animals were not able to see the observer to ensure that its presence did not impact the animal behaviour. Observations were conducted on days 1, 2 and 3 after weaning and/or castration. In detail, CAS-WEA and BUL-WEA were observed on days 1, 2 and 3 of week 0 and CAS-CON and BUL-CON of week 4. The animals were observed for a total duration of 5 h/day consisting of hourly observation periods (0900–1000, 1100–1200, 1300–1400, 1500–1600 and 1700–1800 h). During these 5 observation hours, the number of vocalizations of each group was counted by continuous sampling for 10-min periods. Observations were performed alternately between castrated and bull groups. Thus, each group was monitored for three 10-min periods/day and 15 periods/day.

The calves’ behaviour was recorded by scan sampling at 10-min intervals during the 5 observation hours as listed above. Data were recorded for 15 times/day in each group. The behaviour was classified into feeding, lying, standing and walking (Table 1), though standing and walking were combined and are named standing/walking in the following. In each group, the number of calves performing each of these behaviours was counted at the current sample points. For each of the sampling points, the percentage of calves per group performing the respective behaviours was calculated. In year 1, data were recorded using video monitoring and in year 2 by direct observations. In both years all the vocalization and behavioural observations were recorded by the same person.

Growth performance and slaughter traits
BW of each calf was recorded weekly starting 1 week before until 7 weeks after castration. Average daily gain (ADG) was calculated for 3 weeks following castration, and 3 weeks (weeks 4 to 7) following weaning.

The animals were slaughtered at 17 months of age at a commercial abattoir. BW at slaughter, ADG from birth to slaughter, carcass weight and dressing percentage were recorded. Following slaughter, the carcasses were graded based on the EU beef carcass classification system into the EUROP categories (E = good, O = average, P = poor) and the fat classes (1 = low, 2 = slight, 3 = average, 4 = high or 5 = very high).

Table 1 Ethogram of behavioural observations

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walking</td>
<td>Movement from one location to another without the head orientated at the ground</td>
</tr>
<tr>
<td>Standing</td>
<td>Standing on all four legs without locomotion</td>
</tr>
<tr>
<td>Lying</td>
<td>Lying down with all legs folded under the body and the head down or up</td>
</tr>
<tr>
<td>Feeding</td>
<td>Staying inside the feeding area, which was defined as the area next to the feeder with a rectangular shape; one side as long as the feeder and the other side as long as a calf’s body length</td>
</tr>
</tbody>
</table>
Statistical analysis
The $2 \times 2$ factorial design was analysed using the SAS program, version 9.2 (SAS Institute Inc., Cary, NC, USA). All variables were analysed using the MIXED procedure to test for differences. Data are presented as least square means and pooled standard errors. Multiple comparisons were done by applying the Tukey test with a probability level at $P < 0.05$.

For behaviour traits, the group of calves was defined as experimental unit. Data were analysed considering the following model:

$$y_{ijklm} = \mu + Y_i + W_j + C_k + D_l + W_j \times C_k + W_j \times D_l + C_k \times D_l + e_{ijklm}$$

where $y_{ijklm}$ is the $m$th observation; $\mu$ the overall mean; $Y_i$ the fixed effect of year ($i = 1, 2$); $W_j$ the fixed effect of weaning group ($j = \text{week 0, week 4}$); $C_k$ the fixed effect of castration group ($k = \text{castrates, bulls}$); $D_l$ the fixed effect of day after weaning ($l = 1, 2, 3$); $W_j \times C_k$ the interaction between weaning and castration group; $W_j \times D_l$ the interaction between weaning group and day; $C_k \times D_l$ the interaction between castration group and day; and $e_{ijklm}$ the random residual.

Haematological traits (haptoglobin, percentage of neutrophils and lymphocytes and neutrophil : lymphocyte ratio) were analysed using a repeated measures ANOVA (MIXED procedure) with the individual calf as experimental unit applying the following model:

$$y_{ijklm} = \mu + A_i + W_j + C_k + T_l + W_j \times C_k + W_j \times T_l + C_k \times T_l + e_{ijklm}$$

where $y_{ijklm}$ is the $l$th observation; $\mu$ the overall mean; $A_i$ the fixed effect of year ($i = 1, 2$); $W_j$ the fixed effect of weaning group ($j = \text{week 0, week 4}$); $C_k$ the fixed effect of castration group ($k = \text{castrates, bulls}$); $T_l$ the repeated effect of week ($l = 0$ to 7); $W_j \times C_k$ the interaction between weaning and castration group; $W_j \times T_l$ the interaction between weaning group and week; $C_k \times T_l$ the interaction between castration group and week; and $e_{ijklm}$ the random residual. Because the weaning group $\times$ week and weaning $\times$ castration interactions were not significant for any of the traits, it was deleted from the model. An unstructured covariance matrix was used within animal. The baseline values measured in week 0 were included as a covariate.

Performance data (BW, ADG) were analysed using the MIXED procedure with the fixed effects of year, weaning group, castration group and the interaction between weaning and castration group. Because the castration $\times$ weaning group interaction and the castration group $\times$ week interaction were not significant for any of the traits, they were deleted from the model.

The analysis for the carcass traits (conformation and fat classes 1 to 5) was done with the NPAR1WAY procedure and the treatment groups were compared pairwise with the Wilcoxon rank sum test assuming a probability level of $P < 0.05$.

Results

Behaviour
The number of vocalizations during the first 3 days following weaning was affected by the castration ($P < 0.01$) and weaning group ($P < 0.001$), its interaction ($P < 0.01$) as well as the interaction between castration and day ($P < 0.001$) (Table 2). On day 1 after weaning, the number was higher in the CAS-WEA and BUL-WEA groups than in the CON groups that were weaned in week 4. From days 1 to 3, the vocalizations decreased steadily in all groups and nearly reached zero after this period. Only day affected time spent feeding ($P < 0.05$). Feeding duration decreased from days 1 to 2 and increased again towards day 3 in all groups. The proportion of animals standing/walking was lower on days 1 and 2 in the WEA groups compared with the CON groups. On day 3, the calves of all groups spent about 30% of their time standing/walking. Weaning affected lying duration, too ($P < 0.001$). Groups CAS-CON and BUL-CON spent 20% of the time lying on the 1st day after weaning compared with 40% in CAS-WEA and BUL-WEA animals, though the lying duration increased at a higher rate within 3 days after weaning in the former groups.

Blood traits
Haptoglobin concentrations decreased during the first 5 weeks of the experiment in all groups independent of castration, weaning group or its interaction (Table 3). The proportion of neutrophils and lymphocytes were affected by castration ($P < 0.001$) and weaning group ($P < 0.05$), week ($P < 0.01$) and castration group $\times$ week interaction ($P < 0.01$). In detail, the percentage of neutrophils decreased from the peak in weeks 2 and 3 in CAS-CON and CAS-WEA animals, respectively, whereas in BUL groups the change over time was less pronounced. The proportion of lymphocytes changed vice versa. Castration ($P < 0.05$) and weaning group ($P < 0.05$) as well as week ($P < 0.05$) affected the neutrophil : lymphocyte ratio. Though the change over time was not different between castration and weaning groups, the ratio was greater in CAS when compared with BUL animals ($P < 0.05$) and greater in WEA than in CON calves ($P < 0.05$).

Performance and slaughter traits
ADG during weeks 0 to 3 and weeks 4 to 7 were only affected by weaning ($P < 0.05$). After the WEA groups were separated from their dams, they showed an increased weight gain during weeks 0 to 3 and a reduced weight gain during 4 to 7 weeks in comparison with CON animals that did not respond to weaning with reduced weight gains from weeks 4 to 7. For the ADG from birth to slaughter, none of the effects was significant ($P > 0.05$). At slaughter, bulls were about 80 kg heavier than castrates ($P < 0.05$). The carcass weight differed between castrates and bulls by about 70 kg ($P < 0.05$). Consequently, the dressing percentage of bulls was superior to castrates by 2 to 3% ($P < 0.05$). Castrates were also scored superior in the conformation and fat class than bulls ($P < 0.05$; Table 4).
Discussion

**Behaviour**

Our results showed that the determining factor for the number of vocalizations was weaning and the simultaneous castration and weaning of the animals did not intensify them. Thereby, calves that were already weaned in week 0 were observed with a higher number of calls than animals weaned in week 4. In the later weaned animals, a difference between castrated and intact bulls was not found. As mentioned by Watts et al. (2001), the number of vocalizations depends on several factors such as genotype, sex, age and weight of the animals. In agreement, Smith et al. (2003) found the vocalizations decreasing with the age of the animals at weaning.

During the first 3 days following weaning, the vocalizations were characterized by a significant decrease in all groups. Without considering the effect of castration, Haley et al. (2005) observed a comparable number of vocalizations with 42 calls/h in calves weaned abruptly. Assuming the usefulness of vocalization as a stress indicator, the calves in our study got accustomed to their new social and physical environment after separation from their dams during the course of the 3-day observation period. This was supported by the lying and standing/walking behaviour that changed during the observation period and was affected by weaning. In contrast to our study, Price et al. (2003) also found a decrease in the time spent feeding as an indicator for weaning distress. On day 2, a lower feeding activity was observed compared with days 3 and 4 after weaning conducted with different methods. Although the behavioural measures used in this study, particularly the vocalizations, are valuable indicators for the responses caused by weaning and castration, further studies, including for example serum cortisol measurements are warranted to evaluate the effects of these management procedures in more detail. Again the age effect was determining with the CAS-CON and BUL-CON animals being in better physical condition than calves of the CAS-WEA and BUL-WEA groups. Nevertheless, the older weaned calves might have responded to weaning more distinctly because of their more pronounced mother–calf bond.

Well in agreement with these results, Smith et al. (2003) observed more locomotion in early than in later weaned calves. Enríquez et al. (2010) found the strongest behavioural responses of calves weaned at 6 months of age during the first 2 days after weaning. The high number of vocalizations recorded on days 2 and 3 after weaning in the study of Haley et al. (2005) was accompanied by an increased locomotion activity during these 2 days, too.

**Blood traits**

Elevated concentrations of the acute-phase protein haptoglobin might be induced by weaning and castration. However, effects were not found in the present study. This might be partly due to different sampling times. In agreement with our study, Hickey et al. (2003) did not detect an effect of abrupt weaning on haptoglobin concentration at 1,
2 and 7 days after weaning. Differences in haptoglobin concentrations between ring-castrated and intact calves measured at the same sampling times as in our study were not found by Marti et al. (2010). Conversely, Ting et al. (2005) observed increased haptoglobin concentrations in Burdizzo-castrated calves at 2.5 and 3.5 months of age during the first 7 days following castration, thereafter levels returned to baseline values. An increase in haptoglobin after Burdizzo castration was also reported by Panget al. (2008) during the first 3 days after the treatment.

In response to bacterial infections and stress, neutrophils increase and lymphocytes decrease. A clear indication that weaning or castration induced such changes was not supported by our finding. The castration group × week interaction even indicated a more pronounced decrease of the neutrophil and increase of the lymphocyte proportion in castrated than in intact calves. The course of the neutrophil : lymphocyte ratio did not show different changes during the study period between castration and weaning groups, too. Blanco et al. (2009) weaned calves at 90 or 150 days of age and also did not find an effect of weaning on neutrophil or lymphocyte proportions until 1 week after weaning. A contrary observation was made by Hickey et al. (2003), who found decreased lymphocyte and increased neutrophil proportions as well as increased neutrophil : lymphocyte ratios in weaned compared with control calves until 1 week after the treatment. Panget al. (2009) reported a modest neutrophilia after Burdizzo castration, but no leucocytosis.

Shorter periods between blood samplings may be a possibility to further differentiate the effects of weaning and castration on the blood traits, particularly for the acute-phase protein haptoglobin. Requiring shorter sampling
intervals, the serum cortisol level might be a further possibility to assess the responses to weaning and castration.

**Performance and slaughter traits**

Although weaning affected the weight gain of WEA calves that were weaned at an earlier age than CON animals, the ADG from birth to slaughter did not differ between treatments. An interaction between weaning and castration group was not found (P > 0.05), and thus, weaning is considered to have a more pronounced effect on the performance than castration. The earlier weaned animals compensated for their reduced weight gain from weeks 4 to 7 until slaughter. The results indicate that the nutrient supply was more adequate for the earlier weaned animals immediately after weaning than for the calves that still suckled from weeks 0 to 3. In agreement with our study, Stafford et al. (2002) did not find an effect of Burdizzo castration on weight gain. Observing an effect of Burdizzo castration, Ting et al. (2003) found a lowered ADG of Burdizzo castration on weight gain. Observing an effect of Burdizzo castration, Ting et al. (2003) found a lowered ADG of ring-castrated animals in comparison with intact calves. Burdizzo-castrated calves showed a lower ADG compared with control calves in the study of Mach et al. (2009), too. Contrarily, in 3-month old Holstein calves, Marti et al. (2010) recorded a decreased ADG of ring-castrated animals in comparison with intact bulls during 7 weeks after the treatment. The effects of castration on the slaughter traits found here are comparable to other studies (Keane and Allen, 1998; Kirkland et al., 2006).

In comparison with undertaking the procedures separately, concurrent castration and weaning neither affected behaviour and haematological parameters nor impaired performance. There was no evidence that the concurrent application of both treatments in steer production systems markedly increased the stress response compared with their application at intervals of a few weeks.

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