Weaning and Castration
in Beef Calves

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ABBREVIATIONS

ADG	average daily gain
BW	body weight
CP	crude protein
d	day
DM	dry matter
h	hour
IMF	intramuscular fat
K/µL	10^3 per micro liter
kg	kilogram
kg/d	kilogram per day
km	kilometer
LWG	live weight gain
m	meter
min	minute
MJ ME	megajoule metabolizable energy
MJ NEL	megajoule net energy lactation
µg/ml	microgram per milliliter
rpm	revolutions per minute
T0; T1; …; T5	several sampling points
TP	beginning of pasture period
W6	weaning at 6 months of age
W8	weaning at 8 months of age
WaC	weaning after castration
WBC	white blood cell
WbC	weaning before castration
WsC	weaning simultaneously castration

ABSTRACT

The present work included three trials to investigate the effects of abrupt separation on male and female beef calves of different ages (Trial 1; n=80) and the impact of the timing of this weaning method and the Burdizzo castration on male beef calves (Trial 2; n=32 and Trial 3; n=111). In each trial, vocalization activity, behavior and growth performance of the calves were studied, additionally several blood (Trial 2) and slaughter traits (Trial 2 and 3) were collected. In Trial 1, vocalization and behavior indicated that weaning had a greater impact on the younger weaned calves (6 months of age) compared to calves weaned at 8 months of age. Furthermore performance data suggest to prefer the later weaning age. In Trial 2, treatments were defined either as “weaning conducted simultaneously castration” or “weaning 4 weeks after castration”. Thereby the blood traits, such as Haptoglobin, did not clearly indicate the stressors weaning and castration, potentially due to the sampling times. Castration seemed to reduce vocalization activity, but did not influence behavior after weaning. Although there was an advantage of weaning simultaneously castration in terms of a 3-weeks growth rate after castration, treatment differences disappeared till slaughter. In Trial 3, treatments were defined as either “weaning conducted simultaneously castration” or “weaning 8 weeks before castration”. Results indicated, that vocalizations are induced by weaning rather than by castration. Furthermore, castration reduced vocalization activity and lying behavior. Therefore vocalization activity did not conclusively indicate the well-being status of the calves, when weaning and castration simultaneously occurred. Behavior was influenced by castration and weaning as well as housing conditions. Similar to Trial 2, concerning a short-time growth rate after castration the treatment weaning + castration seems to be preferential, whereas there was no treatment effect on the slaughter traits. Further studies should be executed to analyze the present findings. Thereby stress indicators to be observed should be modified. From the results of this thesis it can be presumed that timing of weaning and castration have to be differentiated for the individual production conditions, concerning housing, feeding and marketing management.

KURZFASSUNG

Die vorliegende Arbeit beinhaltet drei Studien zum Einfluss des direkten Absetzens auf männliche und weibliche Fleischrindkälber unterschiedlichen Absetzalters (Trial 1; n=80) und zum Einfluss von Absetzzeitpunkt und der Burdizzo-Kastration auf männliche Fleischrindkälber (Trial 2; n=32 und Trial 3; n=111). In allen Studien wurden Daten zu Lautäußerungen, zum Verhalten und zur Wachstumsleistung der Kälber erfasst. Außerdem wurden Blut- (Trial 2) und Schlachtparameter (Trial 2 und 3) erhoben. Lautäußerungen und Verhalten in Trial 1 verdeutlichen, dass das Absetzen einen größeren Einfluss auf die jünger abgesetzten Kälber (Alter 6 Monate) hatte, als auf die Kälber, die mit 8 Monaten abgesetzt wurden. Weiterhin lässt sich aus den Wachstumsleistungen eine Empfehlung für das spätere Absetzen ableiten. In Trial 2 wurden die Versuchsvarianten definiert als „Absetzen erfolgt zeitgleich mit Kastration“ und „Absetzen erfolgt 4 Wochen nach Kastration“. Hierbei konnten die betrachteten Blutparameter, auch Haptoglobin, vermutlich auf Grund des Zeitschemas der Probennahme, einen Einfluss von Absetzen und Kastration auf die Kälber nicht eindeutig abbilden. Die Kastration hat die Rufaktivität offensichtlich gemindert, beeinflusste jedoch nicht das Verhalten nach dem Absetzen. Obwohl es hinsichtlich der Wachstumsrate im 3-Wochen-Abschnitt nach der Kastration einen Leistungsvorteil der Variante „Absetzen erfolgt zeitgleich mit Kastration“ gab, verschwanden die Unterschiede zwischen den Versuchsvarianten bis zur Schlachtung. In Trial 3 wurden die Versuchsvarianten definiert als „Absetzen erfolgt zeitgleich mit Kastration“ und „Absetzen erfolgt 8 Wochen vor Kastration“. Die Ergebnisse lassen deutlich werden, dass die Lautäußerungen eher durch das Absetzen als durch die Kastration verursacht wurden. Desweiteren reduzierte die Kastration die Rufaktivität und das Liegeverhalten. Deshalb konnte die Rufaktivität das Wohlbefinden der Kälber nicht schlüssig widerspiegeln, wenn Absetzen und Kastration zeitgleich stattfanden. Das Verhalten wurde von Kastration und Absetzen ebenso beeinflusst wie von den Haltungsbedingungen. Ähnlich wie in Trial 2, scheint die Variante „Absetzen erfolgt zeitgleich mit Kastration“ mit Blick auf die kurzfristige Wachstumsleistung über 3 Wochen nach der Kastration, vorteilhaft zu sein, jedoch gab es keinen Einfluss der Versuchsvariante auf die Schlachtparameter. Zur Absicherung der vorliegenden Ergebnisse sollten weiterführende Studien durchgeführt werden. Dabei sollten die betrachteten Stressindikatoren modifiziert werden. Aus den Ergebnissen dieser Arbeit lässt sich folgern, dass die Zeitpunkte von Absetzen und Kastration auf die individuellen Produktionsbedingungen, wie Haltung, Fütterung und Vermarktung, abzustimmen sind.

SUMMARY

Besides calving, weaning is the most stressful experience in a calf`s life. Abrupt separation is a weaning method characterized by physical separation of mother and young going along necessarily with immediate milk withdrawal for the calves. Depending on the management and production system, additional changes occur in the social and physical environment, as well as in the food supply of the calves at the time of weaning.

When fattened in steer production systems, castration necessarily is conducted on male calves as management procedure. Castration is known to cause a degree of pain and stress, whereas the animal`s response to it is probably dependent on several factors.

Producers know from their experience that the easiest way to reduce stress at weaning is to minimize other management processes undertaken at the time of weaning. However, we are not currently aware of an experimental study concerning the effect of separate weaning conducted in combination with Burdizzo castration on stress responses of beef calves.

The objective of the present thesis is to investigate the effects of the abrupt separation weaning method at different ages on male and female beef calves (Trial 1) and of the timing of abrupt separation and Burdizzo castration on male beef caves (Trial 2 and Trial 3).

In Trial 1, weaning occurred either at 6 (treatment W6) or 8 months of age (treatment W8), in each weaning age 10 male and 10 female beef calves were investigated. Vocalization and three types of behavior (feeding, lying and standing/walking) were recorded from d 1 to d 3 after weaning using instantaneous scan sampling technique. Body weight (BW) of the calves was registered 1 week before weaning, at weaning, 2 weeks after weaning and when the calves of the other treatment were weaned. Average daily gain (ADG) was calculated for 1 week before weaning, the 2-weeks post-weaning period, and the period from 6 to 8 months of age. Live weight gain (LWG) was calculated from birth to 6 and 8 months of age, respectively.

The W6 calves vocalized more often than W8 calves (7.37 vs. 4.29 calls/10min), and female performed a higher number of vocalizations than male calves (6.39 vs. 5.27 calls/10min). Vocalization decreased from d 1 to d 3 after weaning (10.33, 5.43 and 1.73 calls/10min on d 1, 2 and 3, respectively). Feeding behavior was not influenced by the effects in this trial. More W8 than W6 calves (43.8 vs. 39.1%) were lying, whereas the opposite was observed for standing/walking. Lying increased in the course of the days after weaning. The ADG over 2

weeks after weaning did not differ between the weaning ages, but the male calves had a greater ADG than the female calves (0.94 vs. 0.43 kg/d). The W8 calves had a greater ADG from 6 to 8 months of age compared with the W6 calves (0.89 vs. 0.39 kg/d), as well as the LWG, from birth to 8 months, was greater for the W8 than W6 calves (1.07 vs. 0.94 kg/d).
Vocalization activity and behavioral observations indicated that weaning had a greater impact on the younger W6 calves, although there was no effect of weaning age on growth rate up to 2 weeks following weaning. However, the findings of this trial concerning the performance of the calves at 8 months of age as well as the LWG to this age, suggest that the later weaning age should be preferred.

Trial 2 was a 2-year study to evaluate the effects of weaning time relating to Burdizzo castration on blood traits, vocalization, behavior, growth rate, and different slaughter traits. It was conducted on male beef calves of an age of approximately 6.5 months (average BW=253±34 kg). The treatments (each including 8 bulls and 8 castrates per year) were defined as either weaning conducted simultaneously with castration (treatment WsC) or weaning occurring 4 weeks after castration (treatment WaC).
Blood samples for analyzing Haptoglobin, white blood cell counts (WBC), and the number and percentage of neutrophils and lymphocytes were taken from each calf at castration and weekly during the following 5 weeks. Vocalization activity and behavior data (classified as in Trial 1) were recorded from d 1 to d 3 after weaning. BW was measured 1 week before castration, at castration, weekly during the following 5-weeks period, and 7 weeks after castration. ADG was calculated for 1 week before castration, a 3-weeks post-castration period, and a 3-weeks period after weaning the WaC treatment. Additionally, several slaughter traits (e.g. LWG to slaughter, final body weight, carcass weight, EUROP classification, dressing percentage) were recorded.
The WsC calves shouted more often than the WaC calves (3.20 vs. 1.28 calls/10min), castrates vocalized less compared to intact bulls (1.84 vs. 2.64 calls/10min), and the number of vocalizations decreased in the course of the days after weaning. WsC bulls vocalized more than WsC castrates (3.97 vs. 2.43 calls/10min). Irrespective to sex, WsC calves showed a greater number of vocalizations compared to WaC calves. Sex had no impact on behavior after weaning, and treatment did not affect feeding behavior in Trial 2. More WsC calves were lying after weaning than WaC calves (48.0 vs. 38.8%). Day after weaning influenced all types of behavior but the patterns of change were different. For the non-feeding types of behavior there were differences between WsC and WaC calves only on d 1 after weaning. Sex and treatment did not influence Haptoglobin concentrations. However, WBC was

significantly greater in castrates than bulls at 2, 3 and 5 weeks post castration. ADG during 3 weeks following castration was greater for WsC than WaC calves (0.94 vs. 0.68 kg/d) but did not differ between castrates and bulls. ADG during the 3 weeks following weaning of WaC was greater for WaC compared to WsC calves (0.62 vs. 0.10 kg/d). The slaughter traits were not significantly different between the treatments, but showed typical differences between castrates and bulls.

Castration seemed to reduce vocalization activity, but did not influence behavior after weaning. Haptoglobin did not indicate the stressors weaning and castration under the conditions of this trial. Concerning the growth rate during 3 weeks after castration the simultaneous proceeding of weaning and castration had an advantage over weaning after castration. However, treatment had no long-time impact on performance and slaughter traits.

Trial 3 was a 2-year study assessing the impacts of Burdizzo castration and weaning time on vocalization, behavior, growth rates and several slaughter traits in male crossbred beef calves (n=111). Two treatments, each including castrates and intact bulls, were observed in any one year, whereas either weaning and castration occurred simultaneously (treatment WsC) or weaning was executed 8 weeks before castration (treatment WbC).

Vocalization activity was recorded from d 1 to d 3 after castration (year 2 only) and from d 1 to d 3 after castration + weaning (WsC only). Behavior data (according to Trial 1) were collected from d 1 to d 3 after castration. Individual calf BW was measured at castration, 3 weeks after castration and at the beginning of the pasture period. LWG was calculated from birth to castration. ADG was determined for a 3-weeks post-castration period and for the pre-pasture period. Final BW and LWG from birth to slaughter were calculated only in year 1.

Sex did not affect vocalization after castration, whereas WsC vocalized much more than WbC (1.12 vs. 0.01 calls/10min). Castrates vocalized less than intact bulls after castration + weaning (1.64 vs. 2.08 calls/10min). The number of vocalizations significantly decreased in the course of the days. The percentage of calves feeding increased in the course of the days after castration. Less castrates than bulls were lying (15.0 vs. 29.1%) and conversely, more castrates than bulls were standing/walking (62.9 vs. 49.2%). More WbC than WsC calves were standing/walking (57.4 vs. 54.3%) but treatment did not affect lying behavior. ADG during 3 weeks after castration was greater for WsC than WbC calves (1.29 vs. 0.87 kg/d) and lower for the castrates than the bulls (0.93 vs. 1.23 kg/d). ADG of the pre-pasture period did not differ between the treatments. LWG to slaughter was smaller for the castrates than the bulls (0.78 vs. 0.93 kg/d), whereas it was not influenced by treatment.

Results of Trial 3 indicate, that vocalizations are induced by weaning rather than by castration. Furthermore, castration reduced vocalization activity and lying behavior. However, standing/walking was higher when calves were weaned before castration. Weight gains during 3 weeks following castration were improved when castration and weaning occurred simultaneously. This short-time growth performance implies WsC treatment to be preferential. However, there was no treatment effect on the slaughter traits in this trial.

The results confirmed, that in terms of vocalization and behavior responses as well as performance around weaning the older weaning age (8 months) should be preferred. General recommendations for steer producers concerning the time of weaning related to Burdizzo castration cannot be concluded from the findings of the present trials. Vocalization activity did not conclusively indicate the well-being status of the calves, when weaning and castration occurred simultaneously, as castration reduced the number of vocalizations. Behavior was influenced by castration and weaning as well as housing conditions. The blood traits used in Trial 2 did, potentially due to the sampling times, not clearly indicate the impact of weaning and castration on the calves. In terms of performance during a short-time 3-weeks period following castration, the calves that were weaned at castration had an advantage over calves weaned before or after castration. However, treatment differences disappeared up to the slaughter.

Further studies should be executed to analyze the present findings. Thereby stress indicators to be observed should be modified. From the results of this thesis it can be presumed that timing of weaning and castration have to be differentiated for the individual production conditions, concerning housing, feeding and marketing management.

ZUSAMMENFASSUNG

Das Absetzen ist, neben der Geburt, die stressvollste Erfahrung im Leben eines Kalbes. Das direkte Absetzen (abrupt separation) ist eine Absetzmethode, charakterisiert durch die physische Trennung von Mutter und Jungtier, welche mit dem unmittelbaren Milchentzug für das Kalb verbunden ist. In Abhängigkeit von Management und Produktionssystem, treten für das Kalb zum Zeitpunkt des Absetzens zusätzliche Veränderungen auf, sowohl im sozialen und physischen Umfeld als auch bezüglich des Futterangebotes.
Wenn männliche Kälber in Ochsenproduktionssystemen gemästet werden sollen, dann ist für diese Tiere die Kastration eine notwendige Managementmaßnahme. Die Kastration verursacht unbestritten ein gewisses Maß an Stress und Schmerz, wobei die Reaktion des Tieres auf die Kastration von verschiedenen Faktoren abhängig ist.

Tierhalter/innen wissen aus Erfahrung, dass Stress beim Absetzen am einfachsten zu reduzieren ist, indem andere Managementmaßnahmen während dieser Zeit vermieden werden. Der Autorin ist jedoch keine experimentelle Studie bekannt, die sich mit dem Einfluss des direkten Absetzens, durchgeführt in Kombination mit der Burdizzo-Kastration, auf die Stressreaktionen von Fleischrindkälbern befasst.

Ziel der vorliegenden Arbeit ist, den Einfluss des direkten Absetzens auf männliche und weibliche Kälber mit unterschiedlichem Absetzalter (Trial 1) und den Einfluss des Zeitpunktes von direktem Absetzen und der Burdizzo-Kastration auf männliche Fleischrindkälber (Trial 2 und 3) zu untersuchen.

In Trial 1 fand das Absetzen entweder mit 6 (Variante W6) oder 8 Monaten (Variante W8) statt, in jeder Versuchsvariante wurden 10 männliche und 10 weibliche Kälber untersucht. Lautäußerungen und drei Arten von Verhaltensweisen (Fressen, Liegen und Stehen/Gehen) wurden an den Tagen 1 bis 3 nach dem Absetzen, mit der instantaneous-scan-sampling Methode, erfasst. Das Körpergewicht (KG) der Kälber wurde 1 Woche vor dem Absetzen, beim Absetzen, 2 Wochen nach dem Absetzen und beim Absetzen der Kälber der jeweils anderen Versuchsvariante erfasst. Tageszunahmen (TZ) wurden für den Zeitraum von 1 Woche vor dem Absetzen, für die Dauer von 2 Wochen direkt im Anschluss an das Absetzen und den Zeitraum zwischen dem Alter von 6 und 8 Monaten berechnet. Lebendtagszunahmen (LTZ) wurden von Geburt bis 6 Monate sowie von Geburt bis 8 Monate kalkuliert.

Die W6-Kälber riefen häufiger als die W8-Kälber (7.37 vs. 4.29 Rufe/10 min) und die weiblichen äußerten eine größere Anzahl Rufe als die männlichen Kälber (6.39 vs. 5.27 Rufe/10 min). Die Zahl der Lautäußerungen sank von Tag 1 bis Tag 3 nach dem Absetzen (10.33, 5.43 bzw. 1.73 Rufe/10 min an Tag 1, 2 bzw. 3). Das Fressverhalten wurde von den Faktoren in diesem Versuch nicht beeinflusst. Mehr W8- als W6-Kälber (43.8 vs. 39.1 %) lagen, während das Gegenteil für Stehen/Gehen beobachtet wurde. Der Anteil liegender Kälber erhöhte sich im Verlauf der Tage nach dem Absetzen. Die TZ, kalkuliert über den Zeitraum von 2 Wochen nach dem Absetzen, waren zwischen den Kälbern unterschiedlichen Absetzalters nicht verschieden, aber die männlichen Kälber erreichten höhere TZ als die weiblichen Kälber (0.94 vs. 0.43 kg/d). Im Altersabschnitt zwischen 6 und 8 Monaten erzielten die W8-Kälber höhere TZ als die W6-Kälber (0.89 vs. 0.39 kg/d), ebenso waren die LTZ von Geburt bis 8 Monate größer für die W8 als für die W6 (1.07 vs. 0.94 kg/d).
Rufaktivität und Verhaltensweisen verdeutlichen, dass das Absetzen einen größeren Einfluss auf die jüngeren W6-Kälber hatte, obwohl sich das Absetzalter nicht auf die Zuwachsleistung im 2-Wochen-Abschnitt nach dem Absetzen auswirkte. Dennoch lässt sich aus den Ergebnissen dieses Versuches, sowohl bezüglich der Leistung der Kälber im Alter von 8 Monaten als auch mit Blick auf die LTZ bis zu diesem Alter ablesen, dass das spätere Absetzalter bevorzugt werden sollte.

Trial 2 war eine 2-jährige Studie zum Einfluss des Absetzzeitpunktes in Kombination mit der Burdizzo-Kastration auf Blutwerte, Lautäußerung, Verhalten, Wachstum und verschiedene Schlachtparameter. Im Versuch standen männliche Fleischrindkälber im Alter von durchschnittlich 6,5 Monaten (mittleres KG=253±34 kg). Die Versuchsvarianten (jede umfasste 8 Bullen und 8 Kastraten pro Jahr), wurden definiert als „Absetzen erfolgt zeitgleich mit Kastration“ (Variante WsC = weaning simultaneously with castration) und „Absetzen erfolgt 4 Wochen nach Kastration“ (Variante WaC = weaning after castration).
Blutproben für Haptoglobin, Gesamtzahl weiße Blutzellen (WBC) und die Anzahl sowie die prozentualen Anteile von Neutrophilen und Lymphozyten wurden von jedem Kalb bei der Kastration und in den anschließenden 5 Wochen einmal wöchentlich genommen. Daten zu Lautäußerung und Verhalten (vgl. Trial 1) wurden an den Tagen 1 bis 3 nach dem Absetzen erhoben. Das Gewicht der Kälber (KG) wurde 1 Woche vor der Kastration, am Tag der Kastration, einmal wöchentlich während der anschließenden 5-Wochen-Periode und 7 Wochen nach der Kastration erfasst. TZ wurden für 1 Woche vor der Kastration, sowie für die Dauer von 3 Wochen direkt nach der Kastration und für 3 Wochen nach dem Absetzen der WaC-Kälber kalkuliert. Zusätzlich wurden verschiedene Schlachtdaten (z.B. LTZ bis zur

Schlachtung, Mastendgewicht, Schlachtkörpergewicht, EUROP Klassifizierung, Ausschlachtung) erhoben.
Die WsC-Kälber riefen häufiger als die WaC-Kälber (3.20 vs. 1.28 Rufe/10min), die Kastraten riefen weniger als die Bullen (1.84 vs. 2.64 Rufe/10min) und die Anzahl der Rufe nahm im Verlauf der Tage nach dem Absetzen ab. Die WsC-Bullen riefen mehr als die WsC-Kastraten (3.97 vs. 2.43 Rufe/10min). Unabhängig vom Geschlecht äußerten die WsC-Kälber im Vergleich zu den WaC-Kälbern eine größere Anzahl Rufe. Das Geschlecht hatte in Trial 2 keinen Einfluss auf das Verhalten nach dem Absetzen und die Versuchsvariante beeinflusste nicht das Fressverhalten. Es lagen mehr WsC- als WaC-Kälber nach dem Absetzen (48.0 vs. 38.8%). Der Tag nach dem Absetzen beeinflusste alle Verhaltensweisen, jedoch war die Art und Weise der Veränderungen verschieden. Bezüglich der Nicht-Fressverhaltensweisen gab es nur am Tag 1 nach dem Absetzen Unterschiede zwischen WsC- und WaC-Kälbern. Geschlecht und Versuchsvariante beeinflussten nicht die Haptoglobin-Konzentration. WBC war signifikant größer bei den Kastraten als bei den Bullen, gemessen 2, 3 und 5 Wochen nach der Kastration. Die TZ im 3-Wochen-Abschnitt nach der Kastration waren bei den WsC-Kälbern größer als bei den WaC-Kälbern (0.94 vs. 0.68 kg/d), jedoch gab es diesbezüglich keinen Unterschied zwischen Kastraten und Bullen. Die TZ, kalkuliert über 3 Wochen nach dem Absetzen der WaC-Variante, waren für die WaC größer als für die WsC (0.62 vs. 0.10 kg/d). Die Schlachtparameter waren nicht signifikant verschieden zwischen den Versuchsvarianten, jedoch zeigten sich typische Unterschiede zwischen Kastraten und Bullen. Die Kastration hat die Rufaktivität offensichtlich gemindert, beeinflusste jedoch nicht das Verhalten nach dem Absetzen. Haptoglobin hat unter den Bedingungen des vorliegenden Versuches die Stressoren Absetzen und Kastration nicht eindeutig widergespiegelt. Bezüglich der Wachstumsrate im 3-Wochen-Abschnitt nach der Kastration hatte das zeitgleiche Durchführen von Absetzen und Kastration Vorteile gegenüber dem Absetzen nach der Kastration. Jedoch nahm die Versuchsvariante keinen langfristigen Einfluss auf Leistung und Schlachtparameter.

Trial 3 war eine 2-Jahres-Studie, welche den Einfluss von Burdizzo-Kastration und Absetzzeitpunkt auf Lautäußerungen, Verhalten, Wachstum und verschiedene Schlachtparameter männlicher Fleischrindkreuzungskälber (n=111) untersuchte. Jedes Jahr wurden zwei Versuchsvarianten (jede beinhaltete Kastraten und Bullen) betrachtet, wobei entweder Absetzen und Kastration zeitgleich durchgeführt wurden (Variante WsC = weaning simultaneously with castration) oder das Absetzen 8 Wochen vor der Kastration stattfand (Variante WbC = weaning before castration).

Die Rufaktivität der Kälber wurde an den Tagen 1 bis 3 nach der Kastration (nur Jahr 2) und an den Tagen 1 bis 3 nach Kastration + Absetzen (nur Variante WsC) erfasst. Verhaltensdaten (vgl. Trial 1) wurden an den Tagen 1 bis 3 nach der Kastration erhoben. Das KG eines jeden Kalbes wurde am Tag der Kastration, 3 Wochen nach der Kastration und zu Beginn der Weideperiode ermittelt. LTZ wurden von Geburt bis Kastration berechnet. TZ wurden für die 3-wöchige Nach-Kastrations-Periode und für die Vorweidephase kalkuliert. Nur in Jahr 1 wurden Mastendgewicht und LTZ von Geburt bis Schlachtung ermittelt.
Das Geschlecht beeinflusste nicht die Lautäußerungen nach der Kastration, wohingegen die WsC-Kälber sehr viel mehr riefen als die WbC-Kälber (1.12 vs. 0.01 Rufe/10min). Nach Kastration + Absetzen riefen die Kastraten weniger als die Bullen (1.64 vs. 2.08 Rufe/10min). Die Anzahl der Rufe verminderte sich signifikant im Verlauf der Tage. Der Anteil fressender Kälber erhöhte sich im Verlauf der Tage nach der Kastration. Es lagen weniger Kastraten als Bullen (15.0 vs. 29.1%) und andererseits standen/gingen mehr Kastraten als Bullen (62.9 vs. 49.2%). Mehr WbC- als WsC-Kälber standen/gingen (57.4 vs. 54.3%), jedoch beeinflusste die Versuchsvariante nicht die Verhaltensweise Liegen. Die TZ im 3-Wochen-Abschnitt nach der Kastration waren bei den WsC- größer als bei den WbC-Kälbern (1.29 vs. 0.87 kg/d) und bei den Kastraten geringer als bei den Bullen (0.93 vs. 1.23 kg/d). Bezüglich der TZ in der Vorweidephase unterschieden sich die Versuchsvarianten nicht voneinander. Die LTZ bis zur Schlachtung waren bei den Kastraten geringer als bei den Bullen (0.78 vs. 0.93 kg/d), wohingegen dieses Leistungsmerkmal nicht durch die Versuchsvariante beeinflusst wurde. Die Ergebnisse von Trial 3 lassen deutlich werden, dass die Lautäußerungen eher durch das Absetzen als durch die Kastration verursacht wurden. Desweiteren reduzierte die Kastration die Rufaktivität und das Liegeverhalten. Stehen/Gehen wurde vermehrt von Kälbern, die vor der Kastration abgesetzt worden waren, ausgeführt. Die Zuwachsraten im 3-Wochen-Abschnitt nach der Kastration waren höher, wenn Kastration und Absetzen zeitgleich stattfanden. Hinsichtlich dieser kurzfristigen Wachstumsleistung scheint die Variante WsC vorteilhaft zu sein. Jedoch gab es in diesem Versuch keinen Einfluss der Versuchsvarianten auf die Schlachtdaten.

Die Ergebnisse bekräftigen, dass bezüglich Lautäußerungen und Verhaltensreaktionen ebenso wie mit Blick auf die Leistung zeitnah nach dem Absetzen das ältere Absetzalter (8 Monate) bevorzugt werden sollte. Grundlegende Empfehlungen für Ochsenproduzenten, den Absetzzeitpunkt in Kombination mit der Burdizzo-Kastration betreffend, können aus den Erkenntnissen der vorgestellten Versuche nicht abgeleitet werden. Die Rufaktivität konnte das Wohlbefinden der Kälber nicht schlüssig widerspiegeln, wenn Absetzen und Kastration zeitgleich stattfanden, denn durch die Kastration wurde die Anzahl Rufe gemindert. Das Verhalten wurde von Kastration und Absetzen ebenso beeinflusst wie von den Haltungsbedingungen. Die in Trial 2 betrachteten Blutparameter konnten, vermutlich auf Grund des Zeitschemas der Probennahme, einen Einfluss von Absetzen und Kastration auf die Kälber nicht eindeutig abbilden. Hinsichtlich der kurzzeitigen Wachstumsleistung im 3-Wochen-Abschnitt nach der Kastration, hatten diejenigen Kälber, welche zum Zeitpunkt der Kastration abgesetzt wurden, einen Leistungsvorteil gegenüber denjenigen Kälbern, die vor der Kastration abgesetzt worden waren. Jedoch verschwanden die Unterschiede zwischen den Versuchsvarianten bis zur Schlachtung der Tiere.

Zur Absicherung der vorliegenden Ergebnisse sollten weiterführende Studien durchgeführt werden. Dabei sollten die betrachteten Stressindikatoren modifiziert werden. Aus den Ergebnissen dieser Arbeit lässt sich folgern, dass die Zeitpunkte von Absetzen und Kastration auf die individuellen Produktionsbedingungen, wie Haltung, Fütterung und Vermarktung, abzustimmen sind.

1 INTRODUCTION

Artificial weaning, as it predominately occurs in cow-calf production systems, is probably one of the most stressful experiences for a calf. A number of studies have been conducted to assess the impact of weaning on vocalization and behavioral as well as physiological (stress) responses of the calf. An increase in vocalization and time spent walking (Haley, 2006) was found, while time spent eating decreased (Price et al., 2003). Abrupt separation is a weaning method characterized by the separation of mother and young going along necessarily with immediate milk withdrawal for the calves. Depending on the management and production system, additional changes occur in the social and physical environment as well as in the food supply of the calves at the time of weaning.

Castration is well-known to be a pain- and stressful, but essential management procedure performed in beef production systems, in which steers instead of intact male calves are fattened. Several studies were assessed to examine different castration methods, performed at different ages, for their influences on the calf.

In spite of this significant amount of information we are not aware of experimental studies concerning the summarized impact of the abrupt separation weaning strategy and the Burdizzo castration method on beef calves. Therefore, the aim of this thesis is to perform different trials to examine various factors of weaning with concern on calf well-being under field conditions, observed as vocalization, behavior, several blood traits and performance. For evaluating the welfare status, mainly non-invasive methods were used to minimize trial-induced stress. Each of the three trials was executed on another site. Trial 1 included different weaning ages. In the other trials weaning and castration occurred simultaneously as well after (Trial 2) as before castration (Trial 3).

2 LITERATURE SURVEY

“Without stress, there would be no life” (Hans Selye)

2.1 Weaning

Besides calving, weaning is the most stressful experience in a calf`s life. Artificial weaning of beef calves usually is operated by abrupt separation from the dams at a younger age than when natural weaning would occur. According to the European Food Safety Authority (EFSA, 2006) natural weaning of cattle takes place at 8 to 9 months of age. In a semi-wild cattle herd of *Bos indicus*, calves were naturally weaned at an age of 7 to 14 months and the weaning process lasted up to two weeks. In this herd Reinhardt and Reinhardt (1981) found a natural weaning age of 8.8 and 11.3 months for female and male calves, respectively. The weaning process under natural conditions is characterized by gradual reduction of milk intake, while there is an increasing intake in solid food and a change of the mother-offspring bond shown by an increasing social independence of the calf from its dam (Weary et al., 2008).

To measure the attraction of suckler calves to their dams after separation a study using Salers was conducted (Veissier et al., 1990a). Following indoor accommodation during the first three months after birth, the calves were put on the pasture together with their dams, till weaning at 8 months of age. As part of the normal management all calves were used to being separated from the cows for 24 h (Veissier et al., 1990a). In two experiments, choice tests with a total time of two consecutive 5-min intervals were performed on several days after weaning. In such a test the tested calf could choose between either its dam or a familiar herd mate that was either a cow or a calf.

The own dam was longer attractive to the calves (up to d 24 after weaning) when the other choice animal was a cow, compared to the familiar choice calf. In the later test situation calves showed a preference for their dam only up to d 9 after weaning. Most of the calves in this study tried to suck till d 7, but on d 20 only 4 out of 9 tested calves tried to suckle. Furthermore, the observations suggested that the dams` attachment to their calves seemed to decline before the end of the third week after weaning.

The time spent near the choice calf was significantly greater in male than female test calves. When to choose between dam and other cow, the male calves spent a significant lesser proportion of time near their dams compared to the female test calves (Veissier et al., 1990a). The authors concluded that weaning strengthened the bonds between calves. They also presumed that the weaker attachment of the male weaned calves to their dams is due to an older physiological age indicated by the higher growth rates of male compared to female calves.

The effects of weaning on the social organization of the young following weaning was determined by Veissier and Le Neindre (1989) using female Salers calves. The herd splitting into the experimental groups took place when the calves aged about 6 months. The calves of Group 1 were weaned by abrupt remote separation at an age of 7 months. Group 2 consisted of non-weaned control calves. The calves of Group 3 also remained with their dams, but were prevented from suckling due to a cloth on the udder of their dams, from 2 weeks after abrupt separation of Group 1. Observations made in this study were related to several topics according to social behavior. Besides the activity of the animals (lying, standing, moving, grazing, drinking), social encounters were recorded. This encounters were classified into different types of agonistic and non-agonistic encounters. Furthermore, the distance between the animals was observed.
Observation were conducted 2 weeks after herd splitting, 2 weeks after abrupt separation (only Group 1 and 2) and 3 weeks after putting on the upper cloth (only Group 2 and 3). Veissier and Le Neindre (1989) concluded from their results of this experiment that weaning strengthened the social relationships between the calves. They also observed, that the mother-young behavior did not change when suckling was prevented.

Veissier and Le Neindre (1989) conducted a second experiment including Salers heifers weaned at an age of 8 months (Group 1). At weaning, the Salers heifers of Group 1 were placed into a paddock with familiar cows, next to the paddock of Group 2 (suckled control calves remaining with their dams). Three weeks after weaning, the cows in Group 1 were replaced by the respective dams. A surprising finding of this experiment was, that the weaned heifers remained close to each other even after they were reunited with their dams. The authors observed, that there was no significant difference of the behavior of the calves towards their dams, whereas the mothers performed less anti-agonistic encounters with their young

2.1.1 Abrupt Separation

Abrupt separation is a weaning method typically including spatial and visual separation of mother and young going along necessarily with immediate milk withdrawal for the calves. Furthermore, for the young abrupt separation often results in changes of the physical environment (e.g. by getting off the pasture and housing in unacquainted stables, contact with new caretakers), the social environment (e.g. by regrouping and mixing with unfamiliar animals) and food supply (by applying concentrates and silage to pasture reared calves). According to Haley (2006) there are two main behavioral responses to this weaning strategy, firstly, an increase in vocalization and secondly an increase in time spent walking or movement in general when physical space is limited. A decrease in time spent eating and an increase of walking and vocalization as calf`s response after separation were also found by Price et al. (2003) and Veissier et al. (1989). Arthington et al. (2003) reported that acute-phase proteins increased after weaning and transportation. Further details will be provided in the following paragraphs 2.3, 2.4 and 2.5.

2.1.2 Other Weaning Methods

Several studies were conducted to examine the effects of alternative weaning strategies, such as two-step weaning (Boland et al., 2008; Loberg et al., 2008; Haley, 2006; Haley et al., 2005) and fence-line weaning (Boland et al., 2008; Price et al., 2003; Stookey et al., 1997).

The two-step weaning method includes a period of preventing the calf from suckling the dam by fitting an anti-sucking device (nose-flap), while it still remains with the cow (Step 1). This period lasts a few days and is followed by the actual, physical remote separation of the calves from their dams (Step 2).

Fence-line weaning allows the calf to have a further certain social contact to the dam for several days before being totally separated. Due to the fence-line contact, mother and offspring are able to have visual contact and vocal communication, but it prevents tactile contact and therefore access to milk.

2.1.3 Weaning Age

As mentioned before, artificial weaning of cattle mostly is conducted at a younger age as weaning would occur in nature. Producers` decisions according to the weaning time are made independent from the calf using several criteria, such as to improve the physical condition and to increase the pregnancy rate of the cow, to reduce the stocking density on the pasture or to save food. Further reasons can be the capacity available in the fattening housing systems or the supply and demand of beef on the market.

2.1.4 Weaning Age and Vocalization

Crossbred cow-calf pairs (predominately Angus x Simmental) were observed in a weaning study by Smith et al. (2003). The early weaned calves had an average age of 4 months, while the conventionally weaned calves were 7 months of age in average at weaning. For each weaning age two different management strategies after weaning were assed. Thereby the dams either were removed or stayed on the farm after weaning, within visual and auditory proximity to the calves. A further strategy for 7-months old weaned calves was the relocation of the calves to the feedlot immediately after weaning (abruptly weaned treatment). Observation started on the weaning day (d 1) and continued till d 6.
The early weaned calves showed a greater frequency of stress vocalization compared to the conventionally weaned calves. In the course of the days there was a significant decrease in vocalization from d 1 to d 4 in the early weaned, but only from d 1 to d 2 in the older calves. Differences between the weaning ages were found to be significant only on d 2 and d 3 (Smith et al., 2003).
However, irrespective the weaning age, calves showed a greater frequency of stress vocalization in the first three days, when their dams remained within visual and auditory proximity to them. Later, on d 4, these differences between the management groups were less.

In a weaning study on sheep, vocalization activity of lambs directly after weaning was evaluated by giving vocalization scores. Thereby theses scores were highest (intensive and high frequent vocalization) in 6 weeks old lambs, followed by lambs, weaned at 13 and 21 weeks (Schichowski et al., 2010). In that study, latest 5 days after weaning the vocalization activity of the lambs was observed returning to normal values.

In piglets of different weaning ages (3, 4 and 5 weeks of age) Weary and Fraser (1997) found the number of calls declined from 8.2 calls/min in average on the weaning day to only 1.6

calls/min 4 days later. The authors reported that the number of calls was the highest in the youngest weaning age.

2.1.5 Weaning Age and Behavior

Behavioral responses due to weaning at two ages and two different management strategies following weaning were assessed (Smith et al., 2003; details in paragraph 2.1.4). Thereby, the early weaned calves (approximately 4 months old) showed more locomotion (pacing) than the older 7-months old calves.

2.1.6 Weaning Age and Blood Traits

In a study about the acute-phase reaction (details in paragraph 2.5) to an endotoxin challenge Carroll et al. (2009) performed normal weaning at an age of 250 days and early weaning at an age of 80 days on Brahman x Angus calves. After weaning there was one week for acclimatization before the experimental intravenous application of *E. coli* occurred to examine the response of the innate immunity of the calves. Throughout the sampling period that lasted from 2 h before, to 8 h after the application, there was less acute-phase protein Haptoglobin (details in paragraph 2.5) in the blood of the early weaned compared with the normally weaned calves, irrespective of sampling time. However, there was no weaning age x time interaction detected for Haptoglobin concentrations. The authors concluded, that the innate immune system of the early weaned calves appeared to be more competent in reacting to an immune challenge than that of the later weaned calves.

Haptoglobin was used by Arthington et al. (2005) to estimate the stress of early weaned (89 days of age) and normally weaned (300 days of age) steers (Brahman x English) during the 28-days receiving period in the feedlot. Until the time of normal weaning the early weaned calves were kept on the pasture. The transport to the feedlot (about 1,200 km) was conducted for all calves at the time of normal weaning. Blood sampling occurred at the time of normal weaning, on d 1 (arrival at the feedlot) following normal weaning, and on d 3, 7, 14, 21 and 28 of the receiving period. Haptoglobin concentration increased in both weaning ages and was greatest in the normally weaned calves on d 3. Arthington et al. (2005) found Haptoglobin to be negatively associated with the growth rate in the normally weaned, but not in the early weaned calves.

The effect of weaning age (90 days and 150 days) was also examined in a Spanish study undertaken by Blanco et al. (2009a, 2009b). In this study two of the more widespread cattle breeds in the Spanish Pyrenees were involved: the Parda de Montana (selected from the dual-

purpose breed old Brown Swiss) and the autochthonous hardy breed Pirenaica. All calves were housed indoors and, according to the traditional management in the Pyrenees, allowed to suckle their dams for 30 min twice a day during the nursing period. After weaning, the calves remained for 7 days in a barn without any contact to their dams, before being transported to the feedlot.
All calves were blood sampled 168 h before and 6, 24, 48 and 168 h after weaning (Blanco et al., 2009a). The total white blood cell (WBC) counts were not influenced by weaning age at any sampling time after weaning, whereas at the baseline (168 h before weaning) the younger calves had less WBC than the older calves. In this study weaning increased the WBC in the early weaned calves, but 7 days after weaning the WBC numbers were similar to the baseline value. The weaning age did not affect the Neutrophil and Lymphocyte proportion at any sampling time (Blanco et al., 2009a). However, the early weaned calves showed a clear neutrophilia 6 h after weaning, while one week after weaning the baseline value was recovered. Concerning this white blood cell type, the change throughout this study was less evident for the calves weaned at 150 days of age. Blanco et al. (2009a) reported that all leucograms returned towards the baseline values one week after weaning.

Weaning age (150, 210 and 270 d, respectively) had no effect on differential white blood cell counts in Angus x MARC II crossbreds (Bueno et al., 2003). Blood sampling was performed at weaning and afterwards on d 2, 7, 14 and 28 in that study. Also calf performance was assessed, details are shown in the following paragraph.

2.1.7 Weaning Age, Performance and Slaughter Traits

Blanco et al. (2009b) found a higher ADG during the period from 90 to 150 days of age for the early weaned calves (details in paragraph 2.1.6). However, there was no effect of weaning age on slaughter age, carcass weight, fat class, fat color and meat quality, and also no significant interaction between weaning age and breed. During the feedlot period, the early and traditionally weaned calves had similar ADG, whereas the time needed in the feedlot to achieve the target slaughter weight (450 kg) was significantly longer for the early weaned calves (Blanco et al., 2009a).

During the first 28 days after arrival in the feedlot (receiving period), the early weaned calves achieved nearly twice the weight gains than the normally weaned calves, while the feed intake was similar (Arthington et al. 2005; details in paragraph 2.1.6). However, at the end of the receiving period, a tendency for a greater BW in the normally weaned than the early weaned steers was found. Nevertheless, at the end of the 84 days long growing period, that followed the receiving period, no significant difference between the weaning ages appeared. Also the overall feedlot ADG as well as the carcass traits did not differ between the treatments in that study.

Female calves, weaned at 210 days of age had the most rapid post-weaning growth performance, compared to a younger (150 d) as well as to an older (270 d) weaning age (Bueno et al., 2003; details in paragraph 2.1.6). The weaned calves were housed in pens having free access to native grass hay and water, whereas the non-weaned calves were kept on the pasture.

For all calves, weighing occurred 60 days after the weaning of the calves weaned at an age of 210 days. At this time, the calves of the youngest weaning age had been weaned for 120 days and were significantly lighter than the calves weaned at 210 days of age. The calves of the oldest weaning age treatment (270 d) were also lighter at this weighing compared to the 210-days-weaning-age treatment.

However, at the weighing that occurred about 4 months later, the calves weaned at 210 days of age were still heavier compared to the calves weaned at an age of 150 days while they had no performance advantage over the calves of the oldest weaning age in this study (Bueno et al., 2003).

In a study on Simmental x Angus steers the effects of different feeding strategies prior normal weaning (189 days of age in average) were examined. There were two groups with different types of creep feeding and one control group without creep feeding. Additionally, one group of early weaned (63 days of age in average) calves, that were program-fed high concentrates to achieve the same gains as the creep feeding treatments up to the time of normal weaning, was included (Shike et al., 2007). The early weaned calves were housed indoors after weaning while the other calves were grazing on the pasture till weaning.

The authors reported steers showing compensation during a certain period of the complete fattening, consisting of three different periods. Overall, the control calves (normally weaned, without creep feeding) had a 0.12 kg/d smaller ADG and a 29 kg lighter final BW while it took 12 days longer to get them to slaughter compared to the other treatments (Shike et al., 2007). The carcasses of these control steers also achieved lower values. In this study the fattening of early weaned steers resulted to improved carcass quality, but the costs/steer were increased. However, type of creep feeding had no impact on overall performance, carcass quality or carcass value.

There were no significant differences between early (at 4 months of age) and normal (at 7 months of age) weaned calves that remained on the pasture after weaning, concerning ADG during the first and also the second post-weaning week (Smith et al., 2003, details paragraph 2.1.4). In that study a final ADG was calculated from birth to the arrival in the feedlot. Final ADG was significantly lower in the early weaned calves, that had 77 post-weaning days prior to the feedlot, compared to the normally weaned calves having had 14 days between weaning and arrival in the feedlot. The calves of the third treatment, that were abruptly weaned at 7 months of age and transported to the feedlot immediately after weaning, had a greater ADG than the other two treatments mentioned before. However, during the 28-days period after arrival in the feedlot, the ADG was smallest in the abruptly weaned calves and highest for the conventionally weaned calves while it ranged in the middle for the early weaned calves.

Intact male calves of different breeds (Parda de Montana and Pirenaica) and two weaning ages (90 and 150 d) were fed an intensive diet after weaning and slaughtered when a final BW of 450 kg was achieved (Blanco et al., 2009b; details paragraph 2.1.6). ADG, from 90 to 150 days of age, was greater in the early weaned compared to the later weaned calves. Therefore, at weaning of the traditionally weaned calves (d 150), these calves were lighter than the early weaned calves. However, there was no significant difference between the weaning ages for ADG during the finishing phase (d 150 to slaughter).
Nevertheless, there were significant differences between the treatments concerning several parts of the finishing phase. Between d 150 and d 180, weight gains were less in traditionally weaned than in early weaned calves, whereas the former had a greater growth rate between d 210 and d 240. At slaughter, the calves of the older weaning age were 17 days older compared to the calves weaned at 90 days of age (Blanco et al., 2009b). However, feedlot time was 43 days longer for the early weaned calves than for the traditionally weaned calves. Carcass traits as carcass weight and dressing percentage were not affected by weaning age in this study.

2.2 Castration

In a number of studies the castration procedure has been demonstrated to produce behavioral and physiological changes indicative of pain and distress (paragraph 2.3 to 2.5). The results of several studies to assess the differences between castrates and intact bulls indicate in general, that bulls grow faster, utilize feed more efficiently and produce carcasses with less fat content (paragraph 2.6).

The (veterinary) concern about pain caused by castration is not new. Stafford and Mellor (2005) reported, that in 1929, Campbell recommended the second crush of the Burdizzo clamp (paragraph 2.2.3) to occur distal to the first so as to reduce the pain experienced by the animal being castrated. The beginning of direct attempts to assess the levels of pain and distress in farm animals caused by castration is only dated in the 1980s (Stafford and Mellor, 2005).

2.2.1 Steer Production in Germany

The beef production in Germany had been based on the fattening of intact male cattle since the 1950s. In 1912, in Hamburg on the live animal slaughter market, the proportion of castrates was greater compared to intact male cattle (44.7 vs. 12.3%). However, related to that time, the proportion of castrates were halved in 1938, having 15.4% while there were 8.9% bulls. Finally, in 1955, there was a 5 % higher proportion of bulls compared to castrated male cattle on the market. Over all these years, the body weight of the castrates was between 471 and 484 kg, when sold on the market (Winnigstedt, 1957).

In 2009, the total number of slaughtered cattle was more than 3,800,000, while about 40.4% of them were bulls, but only 1.1% were castrates. Thereby, bulls had an average carcass weight of 372 kg and the castrates achieved a carcass weight of 327 kg in average. The most of the 41,694 castrates slaughtered in Germany in 2009, were slaughtered in Lower Saxony (27.5%), nearly as much as that were slaughtered in Bavaria (27.0%) while Schleswig-Holstein was on the third place (14.7%). Furthermore, 2.7% of all castrates that got to slaughter in Germany in 2009, were slaughtered in Thuringia (based on Statistisches Bundesamt, 2010).

In the same year, the monetary value of the beef from castrates, classified as “R3”, was 2.98 Euro/kg carcass weight in average, while the price for the bulls of the same classification was 3.07 Euro/kg carcass weight (BMELV, 2010).

Unlike in Germany, in many other countries it is common to fatten castrated male cattle for beef production. In the United States, more than 17,000,000 bulls are castrated each year, aging between 1 day and 12 months (Lents et al., 2006). Also under the conditions of the northern Australian beef cattle industry, (surgical) castration of males is part of normal management (Petherick, 2005). In Ireland, in 1999, 50% of all cattle slaughtered were castrates and only 2% bulls. In the same year, in the United Kingdom 32% of all slaughtered cattle were castrates and 9% were bulls (Handschur, 2001).

The legal requirements related to cattle castration in Germany are specified in the German Animal Protection Act (Tierschutzgesetz; TierSchG).

2.2.2 Reasons for Steer Production

Two of the most frequently referred reasons for castrate male cattle are probably to reduce aggressiveness, towards human as well as among group housed animals, and to avoid unwanted mating. Some production systems such as common in the United States, cannot work using intact males. According to Comerford (2009) cattle are commingled from many farms numerous times between weaning and slaughter and such commingling of bulls unavoidably result in fighting caused by the need to establish the dominance in the group and can generate injuries and seriously reduce of the carcass value.

Wassmuth et al. (2000) reported that in cow-calf production systems the herd splitting (by calf sex) could be avoided by castration of the male calves while also a long suckling period of about 9 months is enabled. Because of their calm temperament it is less dangerous for the animal keeper to control and handle castrates compared to intact bulls. Whereas less safety arrangements are necessary on the pasture and there are less requirements regarding the housing techniques in steer production systems (Papstein, 1995). Group housing of castrates is not complicated, even commingling with females is possible that could be advantageous for the management.

Steer fattening is common for beef production in Ireland (Keane and Drennan, 2001; Keane and Darby, 2000) and Great Britain (Temisan and Augustini, 1989), where the traditional production systems using castrated male cattle include one or two pasture periods. Also in France, beef production from grass is based on castrated calves (Muller et al., 1991). Ruminants kept in traditional pasture feeding systems often show compensatory growth (Matthes et al., 1983). According to Steinwidder (1996), compensatory growth is the capability of an organism, partly or completely, to gain on a growth deficit (restrictive period) during a following period. This ability is widely studied since Osborne and Mendel (1915 and 1916) reported that growth continued at an accelerated rate after a long period of restriction (Fox et al., 1972). Steinwidder (1996) reported that compensatory growth could be caused by several reasons, like higher feed intake by the compensating animals, improved feed conversion and reduced requirement for maintenance, an increase of the capacity of the digestive tract, and higher protein deposition combined with less fat deposition and therefore a lower deposition of energy.

Through grazing the maintenance and enhancement of grassland biodiversity can be achieved. In a 3-year study, grazing steers of different breeds (German Angus and Simmental) and several stocking densities were observed concerning their impact on the sward (Röver, 2006). The author found, that the steers reacted to changes in sward structure and composition by selective grazing. Through that, the grazing animals created and maintained heterogeneous grassland of possible enhanced biodiversity, while having adequate growth rates on the pasture.

The long-time effects of grazing suckler cows in the high western part of the Thuringian Schiefergebirge (Slate Mountains) had been examine since 1989. During a period of nearly 10 years, the diversity of species was increased by the utilization of the grassland using extensive cutting-grazing regimes. That increase appeared on permanent grassland as well as on sown grassland (Hochberg and Hochberg, 2010). The observed changes of sward composition were due to a combination of low footstep density, regular biting and one annual cutting off the grass. The authors also reported that extensive grazing using cattle or sheep has positive impacts on the diversity of fauna species on the pasture compared to grassland exclusively utilized by cutting.

Beside these advantages in terms of farming management, pasture feeding and biodiversity of grassland, an important argument to produce beef by fattening castrates instead of intact male cattle is the high meat quality of the castrated males. A number of studies were conducted to assess the impact of castration on this topic. At this site only few findings are mentioned.

The chemical composition of the meat is significantly different between castrates and bulls, except the protein content. The content of water and minerals is lower in the meat of castrated than intact males, while the fat content was increased in castrates (Augustini, 1995a). Especially important for the palatability and therefore for the quality of meat is the intra muscular fat (IMF) content (Averdunk et al., 1999). These authors also reported, that there were in castrates 3.2% IMF in the *Musculus longissimus dorsi* compared to 2.5% in bulls of the same breed. Augustini (1995b) reported about intact bulls having an IMF value of 1.16%, that was less than the half of that, found in the castrated males, when all animals were intensively fattened indoors. In castrates IMF content increases as the animals become older (Hühn and Hartung, 1998).

Castrates` carcasses had more subcutaneous fat than non-castrated males, that is beneficial because it reduces the weight loss due to the natural maturing process of the meat after slaughtering (Papstein, 1995).

2.2.3 Castration Methods

All methods of castration are typically characterized by the physical, chemical or hormonal damage of the testicles. Most common are the physical methods, which, according to Stafford and Mellor (2005), can be subdivided into methods in which the testicles are removed surgically by using a scalpel or knife, are damaged irreparably or are killed by elimination of its blood supply. The latter usually is performed by using a Burdizzo clamp, a latex band (Banding method) or elastrator rubber rings.

In surgical castration, the distal scrotum is incised or cut off, than the testicles are removed due to dissection of the spermatic cords or by pulling the cords until they break (Stafford and Mellor, 2005).

The principles of application and function of the latex band and the elastrator rubber rings are similar. Using a special applicator, the rings or bands are placed on the neck of the scrotum, above the testicles and as close as possible to them. By doing this, a better blood-vessel constriction is caused and more of the scrotum is left. Because the rubber ring or band cuts the blood supply to the testicles, the testicles and scrotum degenerate after several weeks and fall off. On calves with larger testicles it is more difficult to apply an elastrator rubber ring so as to be effectively, therefore on these calves often the Banding method is performed.

A Burdizzo clamp is made of high quality steel and has large blunt-jawed pincers. The proper application of the Burdizzo clamp causes the crushing of the spermatic cords and associated blood vessels within the scrotum, while the scrotum remains intact. Therefore there is no open wound on the scrotum, that can become infected, what is the major advantage of this method of surgical castration. Burdizzo castration resulted to an atrophy of the testicles.

Molony et al. (1995) observed the swelling of the castration site after Burdizzo castration to increase slightly over 2 d, from a low baseline level on d 1. However, afterwards the swelling decreased to disappear within 15 d. Ting et al. (2005) examined the effect of calf age (1.5, 2.5, 3.5, 4.5 and 5.5 months) on different responses to Burdizzo castration and found physiological stress and inflammation caused by this castration method indicated reduced temperature differences between the core body and scrotal skin. The authors reported also, that scrotal circumferences increased in all calves on d 1 and d 7 following Burdizzo castration, while there was a markedly greater increase in scrotal circumferences in the 5.5-months old castrates compared to the sham castrated intact bulls of the same age. Similar, Murata (1997) found that the scrotums of 3-4 months old calves rapidly became oedematous after castration and remained swollen during 7 days following castration. In 12-months old steers castrated by using Burdizzo method, on d 14 after castration, greater scrotal latitudinal and longitudinal circumferences were observed than in the intact males of the control group (Pang et al., 2008).

About 120 days after Burdizzo castration, testicles found in Holsteins, aged 8 months at castration, had an average weight of nearly 170 g, while the non-castrated control animals a significant higher testicles weight of 520 g (Mach et al., 2008). In that study, 23% of the castrates (total n=50) did not have a complete testicular atrophy.
Also other experimental studies as well as producers` experience indicate, that the Burdizzo castration method can fail to work. Such failure is often reported to be the major disadvantage of Burdizzo castration, therefore a high level of operator skill and experience is required to complete the usage of the Burdizzo clamp successfully.

The popularity of the methods used for cattle castration varies between management systems and countries (Stafford and Mellor, 2005).

Stafford et al. (2000) published the findings of a survey to identify which castration methods are performed on farms in New Zealand, and at which calf age castration is conducted. According to this survey, 85% of the 2,825 respondents used a rubber ring, 18% castrated surgically, and less than 0.9% used a clamp (Burdizzo castration). Furthermore, 93% of the calves had an age up to 3 months (2.2 months on average), when castrated by the rubber ring method. The surgical castration was performed at an average calf age of 4.3 months, whereas 43% of the calves were castrated during the first 3 months of their life and 39% of the calves were older than 3 months.

A similar survey was directed to cattle farmers in the United Kingdom (Kent et al., 1996). According to their information, the Burdizzo castration was the most performed method (43%), followed by surgical castration (39%) and the castration by using a rubber ring (32%). Furthermore, 10% of the respondents in this survey declared to use more than one castration method. Kent et al. (1996) reported that the surgical castration was most preferred to castrate older calves and 43% of all surgical castration procedures were performed by a veterinarian. 67% of the farmers, who castrated using the Burdizzo method, crushed the cords twice. The majority of producers placed the Burdizzo clamp for the second crush properly distal to the first one.

The results of a survey regarding castration methods directed to bovine veterinarians in the United States, were reported by Coetzee et al. (2010). Over 83% of all respondents (189 veterinarians) quoted that in the practices, the famers were primarily responsible for castration of perinatal calves (BW<90 kg), while 68% of the respondents reported that heavy calves (BW>270 kg) were castrated by a veterinarian. The most frequently used method in perinatal calves was the surgical castration with a scalpel (57%), the most common non-surgical castration was the method using a rubber ring (44%), followed by Banding (22%) and the Burdizzo clamp (21%). Also light calves (BW 90 to 270 kg) were mostly castrated by the surgical method using the scalpel (59%). In light calves, Banding (45%) was the most common non-surgical method, followed by Burdizzo (22%) and elastrator rubber rings (15%). Likewise, the most common method used in heavier calves (BW<270 kg) was the scalpel-used surgical castration (53%), while Banding (51%) and Burdizzo castration (15%) were the two most common non-surgical castration methods.

There is an amount of studies published that examined the effect of the age at castration on the calf. As mentioned before, the recommended ages often vary between the castration methods. It was not the objective of the present thesis to examine different castration ages, therefore findings concerning castration age are not described at this point.

2.3 Vocalization

Vocalization is an useful indicator of welfare, under experimental conditions involving pain or social isolation, if properly used (Watts and Stookey, 2000). The authors assumed, that the call of cattle reflect the biological status of the animal as it interacts with its environment. Therefore vocalization potentially signals the physiological and emotional state, motivations and intentions of the calling animal.

Watts and Stookey (2000) on the one hand declared, that calves vocalize frequently after forced separation and weaning. They also reported, that vocalization may be qualified for indicating pain. Details on both topics are shown in the following paragraphs 2.3.1 and 2.3.2.

In a study to examine vocal responses of cattle 130 calves out of 17 full-sibling families were investigated (Watts et al., 2001a). Observations of the vocalization activity of these calves during a test situation (visual isolation) assumed a partly genetically inheritance regarding vocal characteristics. Also sex, age and weight of the animals were found to influence vocalization. For example, more females vocalized compared to intact bulls during the two observations, that occurred when the animals aged 8-12 month and 11-15 months, respectively. Watts et al. (2001a) observed that vocal animals tended to be older and heavier than nonvocal animals.

In another study, a total of more than 300 newly weaned steers of 4 phenotypic breed groups were observed during routine processing and visual isolation (Watts, 2001). Depending on their coat color, the animals were designated as either “pure” Angus, “pure” Charolais, crossbred Angus or crossbred Charolais. Thereby, all of the 72 crossbreds had an appearance typically caused by the influence of the white-head cattle breed Hereford. The phenotypical “pure” Angus were vocalizing significant more than “pure” Charolais, when restraint for normal management handling (e.g. ear tagging and implanting). When observed during visual isolation for 1 min, the Angus type cattle tended to vocalize more than the cattle of Charolais type.

Watts (2001) concluded from his studies, that vocalization of cattle in testing environments was influenced by genotype, phenotype, age, weight, sex, gender and presence and activities of companions. Therefore, these factors have to be controlled as they could confound the effects of the observation interest. He also preferred to use vocalization measurements alongside other measurements to assess welfare status of groups, not individuals.

2.3.1 Weaning and Vocalization

Several studies were conducted, wherein the vocalization of calves after weaning was observed to assess the welfare status of the young. In those studies, mostly different weaning strategies are included, but non-weaned control groups are rarely involved.

The abrupt separation and the two-step weaning were performed at an average weaning age of 187 days (Haley et al., 2005). The abrupt weaned calves were observed to produce 41.9 calls/h on d 2 and d 3 following separation from the dams.

The two-step weaning method was also performed in a study using sheep of two breeds (Schichowski et al., 2008). Weaning was performed at different weaning ages (8 and 16 weeks of age), the control groups consisted of abruptly separated lambs. For the two-step weaned lambs, the prevention of the lambs from suckling while still remained with their mothers was realized by a net covering the ewes` udder (Step 1). The remote separation of these lambs (Step 2) occurred one week following Step 1. The separated lambs and their ewes were housed in different barns as to prohibit visual contact as well as vocal communication. Observations were made from 4 days before to 4 days after weaning for 3 h/d, whereas the total number of bleats coming from each lamb was counted.
No vocalization activity was found before weaning and after d 2 following weaning. The number of bleats was significant different between the breeds (Rhoensheep and German Merino) and higher in the abruptly weaned compared to the two-step weaned lambs. In this study, the greatest number of bleats was observed in the younger lambs weaned by abrupt separation (Schichowski et al., 2008).

Offspring from Angus and Angus x Hereford cows was examined in a study including abrupt weaning, fence-line and two-step weaning method (Enríquez et al., 2010). Abrupt separation occurred on d 0 while the fence-line separation and application of the anti-suckling devices (nose-flaps), respectively, were conducted 17 days before d 0. For all calves the remote physical separation from the dams was performed on d 0, at an average calf age of 180 days. In that study, the overall vocalization activity was significantly higher in the fence-line weaned calves than in the abruptly and the two-step weaned calves.

More findings about vocalization activity from studies performing the abrupt separation method with concern on the weaning age are shown in paragraph 2.1.4.

2.3.2 Castration and Vocalization

Several studies with concern on castration and vocalization were conducted on different farm animal species as cattle, sheep and pigs. Further information on an assortment of these studies are shown below.

However, studies on pigs predominately observed vocalization during the immediate castration process. For example, Puppe et al. (2005) and Schön et al. (2006) observed vocalization behavior during three periods of the surgical castration of piglets (pre-surgical handling, surgery and post-surgical handling). Taylor and Weary (2000) also examined in detail the vocalization activity of piglets regarding the different components of the castration procedure (restraint, washing the ano-genital region, scrotal incision and pulling/severing of the spermatic cords). The aim of White et al. (1995) as well as of Sutherland et al. (2010) was to examine the vocalization behavior of piglets during castration with or without local anesthesia.

Behavioral observations were made on lambs over 10 weeks of age to evaluate the acute and long-term effects of castration (Melches et al., 2007). Three different castration methods were included (Burdizzo, rubber ring and surgical castration), whereas all castrations were performed under local anesthesia. Vocalization was recorded throughout the castration procedure and the handling of the non-castrated control lambs, respectively. Further observation sessions took place on several days up to d 30 following castration in all lambs and several times during a period of additional 30 days in lambs, when the castration wound was not completely healed one month after castration. Vocalization was included in a combined index “total activity” as well as behaviors classified as foot stamping/kicking, easing quarters, standing up/lying down and head turning (Melches et al., 2007).

Vocalization during surgical castration was observed in cattle to identify and quantify pain by Currah et al. (2009). Coetzee et al. (2008) also determined vocalization at time of castration or simulated castration in cattle.

2.4 Behavior

2.4.1 Weaning and Behavior

The behavior of heifers (about 7 months of age) after the separation from their dams and the transport from the pasture to indoor pens, was examined by Veissier et al. (1989). The animals were Salers and Aubrac, both suckled by their own dams, Friesian suckled by Salers foster cows and artificial reared Friesian heifers. Observations were made on d 1, 2, 4, 6, 9 and 12 following weaning. From d 1 to d 12, the authors found the time spent lying or "resting chin" increased from an average of 30 to 58%, while time spent standing or moving decreased from an average of 49 to 21%. There was a decline in the number of bouts of any behavior pattern. The time spent feeding did not differ between the groups, but the Friesians were less active than the Salers and the Aubracs, whereas the Aubracs remained more active than the Salers until the end of the experiment. The artificial reared Friesians seemed fully adapt on their new environment by d 2 of this study, while for the other animals the stabilization did not occur before d 4. For the latter the weaning procedure was performed as an extra occurrence in addition to transport and housing (Veissier et al., 1989).

When the abrupt weaning method, used as control, was compared to the two-step weaning, a higher locomotion activity was observed in calves weaned by the former strategy. Haley et al. (2005) applied in their study a calf stride length of 65 cm. They reported that control calves walked an average of 5.8 km/d more than the two-step weaned calves on the first 4 days after separation. On d 1 after separation, about 11.5 km/d more were walked by the control calves compared to the calves of the other weaning method.

2.4.2 Castration and Behavior

In a frequently citied study, different castration methods (Burdizzo, rubber rind, surgical castration) were performed on very young Ayrshire bull calves, at an age of 6, 21 or 42 days (Robertson et al., 1994). The study included also handled male calves, that remained intact bulls. During 180 min following castration, active behaviors and posture of the calves were observed. Thereby observed behaviors were foot stamping, tail wagging, head turning, eating and ruminating. Furthermore, the number of times a calf stood up and lay down (restless score) were recorded, and the lying and standing postures showed by the calves were classified. After castration by the Burdizzo castration method, abnormal standing of the calves consisted mainly of 'statue' standing, a stationary posture possibly adopted to minimize pain (Robertson et al., 1994). In the 21-days old Burdizzo castrated calves an increased tail wagging was found, while other behaviors were not significantly different between handled intact bulls, surgical and Burdizzo castrated males. From the responses found in this study, the authors presumed that all castration methods caused pain, irrespective of age at castration, whereas the Burdizzo castration appeared to cause the least pain.

Some other studies assessed the impact of castration on calves` behavior and postures, whereas most of them did not perform the Burdizzo castration. However, there were immobile postures due to both, surgical and Burdizzo castration, in one-week old Ayrshire calves (Molony et al., 1995). Calves behavior and postures were recorded throughout a total period of 48 days after castration. When calves were castrated by using the Burdizzo method, there was an increased statue standing during the first 180 min following castration. During this observation period, in general, time spent abnormal standing increased in castrates compared to intact male control calves (Molony et al., 1995). However, time spent abnormal lying, normal lying and total lying, respectively, did not significantly differ between Burdizzo castrates and control calves during the first 180 min after castration.

In that study, no significant differences between the treatment groups (8 calves/treatment) were found in terms of time spent eating, ruminating, total standing or playing. However, time spent playing decreased during the time of observation, while time spent for the other behaviors increased (Molony et al., 1995).

2.5 Blood Traits

2.5.1 Acute-phase Response and Haptoglobin

The acute-phase response (APR) is a non-specific and early-defense mechanism of the host activated by imbalance of homeostasis due to tissue damage, infection, stress, or inflammation (Cray et al., 2009; Orro et al., 2008). Alsemgeest (1994) found in viral infection, generally the APR to be milder, while bacterial infections usually lead to a strong systemic acute-phase response (citied in Gruys et al., 2005). Acute-phase proteins are synthesized by liver cells as part of the acute-phase response. Haptoglobin is a major acute-phase protein in cattle and is considered a good candidate for monitoring stress responses in this species (Slocombe and Colditz, 2004). Major acute-phase proteins are characterized by a very low concentration in healthy animals as well as a very small half-life period. Haptoglobin belongs to the group of positive acute-phase-proteins, that plasma concentration increase during the APR. The physiological value of the Haptoglobin concentration in the blood of cattle is neglectably or even below the detectable value (Uchida et al., 1993, cited in Chan et al., 2004; Bremner, 1964, cited in Kaepke, 2002). According to Panndorf et al. (1976) a physiological Haptoglobin concentration is up to 0.1 mg/ml (cited in Kaepke, 2002). Richter (1974) reported hardy detectable changes of Haptoglobin in healthy cattle and found Haptoglobin not to be influenced by sex, age, milk yield or pregnancy (cited in Kaepke, 2002). In a Taiwanese 1-year study conducted examined young females, no effect of environmental temperature changes on Haptoglobin as well as differences between individuals were found (Chan et al., 2004). Furthermore, these authors reported that the Haptoglobin concentrations did not differ when recorded before or following a pregnancy.

According to Slocombe and Colditz (2004) serum Haptoglobin can increase 100 fold in cattle in response to stressful stimulus as weaning, transport, mixing unfamiliar cattle and the feedlot environment. Connor et al. (1988) found a 100 fold increase of Haptoglobin concentrations within a 24-hour period following induced local inflammation (cited in Chan et al., 2004) In this study, oil of turpentine was subcutaneously injected in calves. Caused by this, Haptoglobin concentration increased, whereas the Haptoglobin-levels varied with the injected dose. In a study with clinical ill cattle Horadagoda et al. (1999) found Haptoglobin having the highest clinical specificity of 76% in discriminating between acute and chronic inflammatory conditions (citied in Cray et al., 2009).

2.5.2 Weaning and Haptoglobin

About the effects of the abrupt weaning strategy on 7-months age male and female crossbred beef cattle was reported by Hickey et al. (2003). This study included the weaned calves as treatments and non-weaned calves as control groups. It has to be pointed out that all calves in this study were used to the handling (blood sampling) procedure, sampling times were 168 h before weaning (baseline), and 6 h (male calves only), 24, 24 and 168 h after weaning. Plasma Haptoglobin concentration was not influenced by weaning, sampling time or sex (Hickey et al., 2003).

Differing findings were reported by Lynch et al. (2010). In this study, also including weaned and non-weaned calves (details in paragraph 2.5.5), sampling time was significant for Haptoglobin, with an increase on d 2 after weaning compared to the baseline value. However, treatment did not influence Haptoglobin concentration.

In a study including two experiments, the Haptoglobin concentration in newly weaned Brahman x Angus calves increased over the post-weaning time, only in Exp. 2 (Arthington et al., 2003). In Exp. 2, the effects of the additional incidents transportation (for 3 h) and commingling on newly weaned female and castrated male calves (average BW of 222 kg) were researched. Blood sampling occurred at weaning and on d 1, 5, 9, 13, 17 , 21 after transport. Haptoglobin was not affect by calf sex in this experiment. The Haptoglobin concentration was highest on d 5 after weaning, while on d 9, 13 and 21 following weaning, it was lower and also not significantly different from the weaning value. On d 17 after weaning, Haptoglobin concentration was higher than at weaning, but lower than on d 5 while it did not significantly differ from the value on d 21. Because of the absence of a non-weaned control group it was not possible to determine if the increase of Haptoglobin was directly caused by weaning or may be by another inflammatory stressor (Arthington et al., 2003). However, the results indicated that the APR in the newly weaned calves was influenced by transportation associated stressors.

2.5.3 Castration and Haptoglobin

Surgical castration was conducted on 6- to 9-months old calves in a study published by Faulkner et al. (1992). The authors assumed that the acute-phase protein Haptoglobin may be a more specific indicator of inflammatory process triggered by the injury of castration than cortisol, that would be an indicator of the whole body stress response.

In a study published by Ting et al. (2005) the Burdizzo method was performed in Holstein calves of different ages (1.5, 2.5, 3.5, 4.5 and 5.5 months). The study included 5.5-months old intact male calves, which were shame castrated. Blood samples for Haptoglobin were taken before castration on d 0, and afterwards on d 1, 3, 7, 14, 21, 28 and 35. Following castration there was an interaction between treatment and sampling time detected. On d 1 and d 3 after castration Haptoglobin concentration was greater in the 5.5-months old castrates compared to the intact bulls. Haptoglobin concentration due to castration was greater in the oldest castrates (5.5 months) compared to calves castrated at younger ages (1.5 and 2.5-months, respectively). However, irrespective of the castration age the Haptoglobin concentrations returned to the baseline value (d 0) from d 7 onwards (Ting et al., 2005).

In a similar study concerning the age of the calves and their weaning status at castration, respectively, was reported by Lyons-Johnson (1998). Calves, which were castrated aging 36 weeks (8.4 months) at weaning had higher Haptoglobin concentrations than 33-weeks old calves, castrated 3 weeks before weaning. However, the castration methods performed in this study were surgical castration and Banding.

2.5.4 Leukocyte Population

The following definitions are published by www.merckvetmanual.com (2010). According to that, the white blood cells (WBC) in the blood of mammalians included segmented neutrophils, band neutrophils, lymphocytes, monocytes, eosinophils and basophils. WBC are also named leukocytes. Leukocytosis is an increase in the total number of circulating WBC, while leucopenia is a decrease. Differential WBC counts can be reported either as total cell numbers per volume of blood (µL) or in relative percentages of the total.

2.5.5 Weaning and Leukocyte Population

Weaning and sex did not affect the total WBC in the blood of 7-months old beef calves, while weaning increased the number of leukocytes 24 and 48 h after the separation and also decreased the lymphocyte proportion on d 1, 2 and 7 following weaning (Hickey et al., 2003; details in paragraph 2.5.2). In this study there was a weaning x sampling time interaction found for neutrophils, showing an increase on d 1 and d 7 after weaning. Interestingly, the male calves had a higher neutrophils proportion than the female calves (Hickey et al., 2003).

In a recently published study abruptly weaned and non-weaned steers were involved to assess the effect of abrupt weaning at housing on several blood traits including leukocytes and acute-phase proteins (Lynch et al., 2010). In this study weaning occurred simultaneously with housing, at an average age of 235 days and an average body weight of 310 kg. Blood samples were taken 1 week before weaning/housing, at weaning and on d 2, 7 and 14 following weaning. The authors found an significant increase of the total leukocyte numbers on d 2 compared to the other sampling times, the number of neutrophils also was increased on d 2, whereas the number of Lymphocyte was decreased at this time. All these blood traits did not change in the non-weaned controls during the complete sampling period. Therefore, on d 2 after weaning, the total leukocyte number, the number of neutrophils and lymphocytes was significantly greater in the weaned compared to the non-weaned calves.

2.5.6 Castration and Leukocyte Population

Following surgical castration of 5-months old bull calves Fisher et al. (1997) found an increase in the total WBC counts, that was mainly caused by the increasing number of neutrophils. Murata (1997) found leukocytosis primarily due to neutrophilia following Burdizzo castration in 3-4 months old Holstein calves. WBC was significantly higher on d 2 after castration compared to the pre-castration value. However, the WBC counts on d 7 post castration were not different from that measured just before castration. In this study, lymphocyte numbers did not change during an observation period of 7 days after castration, whereas a decrease of the T-Lymphocytes population in Burdizzo castrated calves was found by Murata and Takahashi (1998).

Ting et al. (2005) (details paragraph 2.5.3) found on d 2 after Burdizzo castration a significant greater WBC number in the 5.5-months old castrates than in the intact bulls of the same age. Furthermore, on d 2, the amount of WBC was greater relative to the values measured on d 1, in all castrates except the group of the calves, castrated at 3.5 months of age. The WBC number of the 1.5-months old castrates was greater on d 7, 14 and 35 in comparison with the 5.5-months old castrates. Furthermore, on d 28 of castration of this study the youngest castrates (1.5 months) had a significant greater WBC number compared with all other castrates (Ting et al., 2005).

2.6 Performance

2.6.1 Weaning and Performance

In a study on 7 months old heifers Veissier et al. (1989) (details in paragraph 2.4.1) calculated the growth rate from d 0 to d 11 after weaning. It was highest in the artificial reared Friesians (1.6 kg/d), and higher in the fostered Friesians (1.1 kg/d) compared to the Aubracs (0.6 kg/d), but it was not different between Salers (0.7 kg/d) and Aubracs (0.6 kg/d).

In newly weaned calves Arthington et al. (2003) found for a 7-days period following weaning a BW loss of -3.4% when calves were transported immediately after weaning, while the non-transported calves had a BW gain of 0.7%. In a second experiment of this study, the growth rate of the calves were calculated for a 21-days post-weaning period. In that, both treatments had a BW loss, whereas the BW change was greater in the non-transported than the transported calves (-0.4 vs. -2.4%). The authors supposed that the growth rate differences between the experiments were due the length of data collection.

2.6.2 Castration and Performance

The effects of the pre-pubertal Burdizzo castration, performed on 8-months old Holstein bulls, on performance, carcass and meat quality were evaluated (Mach et al., 2008). At castration an anti-inflammatory via intramuscularly was injected to the animals, and they were fed concentrate and barley straw, both *ad libitum* for 121 days following castration, before they got slaughtered. In this study, including 50 intact bulls and 50 castrates, the ADG and the final BW were greater for bulls than castrates. However, the concentrate intake was not influenced by castration in this study, just as castration did not affect carcass conformation (89% classified as “O”). However, the carcasses classification for degree of finishing (fat class) was greater in the castrates (92.9% classified as “3”) compared to the bulls (68% classified as “3” and 32% classified as “2”).

In contrast, in calves aging from 1.5 to 5.5 months there was no effect of the Burdizzo castration on the growth rates, calculated over a 42-days period from 1 week before castration to d 35 after castration (Ting et al., 2005; details in paragraph 2.5.3).

In implanted calves, either surgically castrated at 70, 230 or 410 kg or left intact, no significant differences in feed efficiency were found between the castrates (Worrell et al., 1987). On all calves, the same implanting regime was applied. However, the intact bulls were more efficient in converting feed and had a higher ADG compared to the castrates. The ADG during the growing phase (lasted 84 days, up to 320 kg,) was decreased in animals castrated at the beginning of this period compared to intact bulls and males castrated at 420 kg. Furthermore, the castration at 320 kg (beginning of the 112-days finishing period) resulted in smaller weight gains during the following final fattening than non-castration and castration at 70 or 230 kg, respectively (Worrell et al., 1987).

Fisher et al. (1996) performed different castration methods with or without local anesthesia on 5.5 months old Friesian calves. The authors found ADG during one week following castration to be significantly lower in the calves, castrated by Burdizzo with local anesthesia, compared to the intact control bull calves. However, ADG during this first week after castration was not different between the Burdizzo-castrates with and without local anesthesia. During the second and also fourth week following the castration no growth rate difference between the treatments was found, whereas during the third week only Burdizzo-castrates without local anesthesia achieved significant less ADG compared to the intact bull calves.

Holstein-Friesian steers, castrated at 6 months of age, and bulls of the same breed were evaluated in a study by Kirkland et al. (2006), with regard to the effect of slaughter weight on animal performance. Besides others, two treatments comprised bulls and steers with slaughter live weight gains of 450 kg. The ration of these treatment animals consisted of *ad libitum* concentrates and restricted barley straw. Although the daily intake was not different between bulls and steers, the latter required significantly more dry matter than the bulls to achieve the equivalent slaughter weight. Kirkland et al. (2006) found the castrates having significantly lower growth rates and to be significantly older at slaughter compared to the intact bulls of the same slaughter weight. However, dressing percentage, carcass conformation and also carcass fat classification were not significantly different between the sexes.

In a study by Keane and Allen (1998), also conducted with concern on several final body weights at slaughter, Friesians and Friesian crossbreds with Limousin and Belgian Blue, respectively, were included. Steers were castrated at 2 months of age, the rearing management for bulls and steers was the same up to about one year of age. The following diet was mixed of concentrates and grass silage, either offered *ad libitum* or as 80% of *ad libitum* intake. Keane and Allen (1998) found significantly greater live weight gains for the bulls than for the castrates.

3 MATERIALS AND METHODS

3.1 Trial 1 - Influence of sex and age on behavior and performance in beef calves after weaning.

3.1.1 General

The location of Trial 1 was the experimental farm "Rudlos" of the Department of Animal Breeding and Genetics of the University of Giessen, Hesse, Germany. Rudlos is located in the low mountain range Vogelsberg with an average height of 400 m above sea level and an average annual rainfall of 500 mm. The mean temperature in this region is 7.5 °C.

3.1.2 Animals, Treatments and Management

A total of 80 calves consisting of 23 Angus and 57 crossbreds (Angus x Simmental, n=19 and Simmental x Angus, n=38) from the suckler cow herd managed at Rudlos were involved. The trial included two successive replications of the same experimental design. In replication 1 February-born calves (n=40) were used, in replication 2 March-born calves (n=40) from the same year were examined. A total of 42 calves were offspring of 5 Angus bulls (3 to 16 calves/bull), and 38 calves were sired by 4 Simmental bulls (4 to 12 calves/bull).

A 2 x 2 factorial design was conducted to determine the effects of weaning age and sex of the calves on their behavioral stress response and performance after weaning. The treatments were derived from two weaning ages:

1) 6 months of age (W6),
2) 8 months of age (W8).

The W6 calves (n=40) were weaned at 192 (±5) days of age with an average body weight (BW) of 249.3 (±27.3) kg. The W8 calves (n=40) were weaned at 241 (±5) days of age, with 300.6 (±35.8) kg BW in average. In each treatment 10 male and 10 female calves were observed.

Table 3.1 Trial 1 - Number of calves by genetic group, replication, weaning age and sex

Weaning age	Sex	Replication 1 A[1]	A x S	S x A	Total	Replication 2 A	A x S	S x A	Total
6 months	Male	3	3	4	10	3	3	4	10
	Female	2	2	6	10	4	2	4	10
8 months	Male	3	2	5	10	3	3	4	10
	Female	2	2	6	10	3	2	5	10
Total		10	9	21	40	13	10	17	40

[1] A = Angus; A x S = Angus bull x Simmental dam; S x A = Simmental bull x Angus dam

One week before weaning the cow-calf pairs were placed into a straw bedded pen in an open-front barn. So the calves of each treatment group could get accustomed to each other. In the morning of the day of weaning the calves were weighed and separated from their dams. The calves were accommodated in a closed part of the barn within earshot to their dams but without visual or physical contact to them. The groups of male and female weaned calves were housed in equivalent straw bedded pens adjacent to each other (3.6 m^2/calf). The calves of the two groups were able to have tactile contact to each other. The calves were fed with a daily ration consisting of a mixture of grass silage (6.0 kg; 2400 g DM), corn silage (8.5 kg; 2800 g DM) and spent grain (1.0 kg; 260 g DM) and additional hay (0.5 kg; 340 g DM). The mixture was offered in troughs hanging on the front of the pens (1.9 m trough length; 2 troughs/pen) while the hay was put on the floor. In total there was one feeding site for each calf. Water supply was provided for self-watering bowls, one bowl per pen.

Two weeks after weaning, the W6 calves moved into a separate solid stable for a following 5-week period. Therein calves were bedded on straw, had free access to water and bales of grass silage.

3.1.3 Vocalization and Behavioral Observations

Direct observations were performed to determine the calves` behavior and vocalization on d 1, 2 and 3 after weaning. The animals were observed 5 h/d (0800-0900, 1000-1100, 1200-1300, 1400-1500 and 1600-1700).

Instantaneous scan sampling technique with a 5-min time period was used to count the total number of vocalizations coming from each group. Observations were performed alternately between the two sex groups. For each group there was a total observation time of 30 min/h and 150 min/d, respectively. For comparability reasons the counts of two following 5-min periods were summarized and the number of vocalizations per calf in a 10-min period was calculated.

The calves` behavior was classified as one of three types of behavior: feeding, lying, and standing/walking. In the present trial, no differentiation was made between standing (without locomotion) and walking (as type of locomotion) behavior of the calves because of the design of the pens and the observer`s location. Behavior was classified as feeding when the calf was standing inside the feeding area, as this behavior normally is performed in the context of food consumption. Putnam et al. (1968) found 94 to 97% of time spent standing at the feeder was spent feeding. For this thesis, the feeding area 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.

In each group, the number of calves engaging in each of these types of behavior was counted at the current sample points, on a rotating basis, and percentage of calves per group was calculated. For each group there were 6 sample points/h and 30 sample points/d, respectively.

3.1.4 Growth Performance

Individual body weight (BW) data of the calves, measured in kilogram (kg), were collected 1 week before weaning, on the day of weaning and 2 weeks after weaning. Additionally the calves of each treatment were weighed when the calves of the other treatment were weaned. Thereby for each calf a BW at 6 and 8 months of age was recorded.

Live weight gain (LWG), measured in kilogram per day (kg/d), was calculated from birth to 6 and 8 months of age, respectively. Average daily gain (ADG), measured in kilogram per day (kg/d), was calculated for the 1-week period before weaning, the 2-weeks period following weaning and for the 7-weeks period between the BW measurements at 6 and 8 months of age.

3.1.5 Statistical Analysis

All statistical analyses were conducted using the SAS program (Version 9.2; SAS Institute, 2008). Data are presented as least square means (lsmeans) with subscript standard errors (s.e.). Interactions between effects were removed from the model when tested to be non-significant ($p \geq 0.05$). Multiple comparisons were done by applying the Tukey`s test (alpha=0.05).

For the analysis of the group recorded data (vocalization and behavior) the GLIMMIX procedure was used with a model including replication (1 and 2), weaning age (6 months=W6 and 8 months=W8), sex (male and female), and day after weaning (d 1, 2 and 3) as fixed main effects. Vocalization activity was analyzed considering the following model:

$$y_{ijklm} = \mu + R_i + W_j + S_k + D_l + W_j \text{ x } S_k + W_j \text{ x } D_l + S_k \text{ x } D_l + R_i \text{ x } W_j \text{ x } S_k + e_{ijklm},$$

where y_{ijklm}=the mth observation; μ=overall mean; R_i=fixed effect of replication (i=1, 2); W_j=fixed effect of weaning age (j=6 months, 8 months); S_k=fixed effect of sex (k=male, female); D_l=fixed effect of day after weaning (l=1, 2, 3); W_j x S_k=interaction between weaning age and sex; W_j x D_l=interaction between weaning age and day after weaning; S_k x

D_l=interaction between sex and day after weaning; R_i x W_j x S_k=three-way interaction between replication, weaning age and sex, and e_{ijklm}=random residual.

The model for analyzing feeding behavior included only the fixed main effects, described before, and e_{ijklm}=random residual. The behavior types lying and standing/walking were analyzed using a model that included the main effects and additional R_i x D_l=interaction between replication and day after weaning; W_j x D_l=interaction between weaning age and day after weaning; and e_{ijklm}=random residual.

For analysis of performance data (BW and ADG) the GLIMMIX procedure was used. The model included the effects of replication (1 and 2), weaning age (6 months=W6 and 8 months=W8), and sex (male and female) as fixed main effects and additional the significant interactions between them. For performance data individual calf served as the experimental unit. The model for BW data, LWG to 6 months of age, ADG for 1 week before and ADG 2 weeks after weaning was as follows:

$$y_{ijkl}=\mu + R_i + W_j + S_k + R_i \times W_j + e_{ijkl},$$

where y_{ijkl}=the l^{th} observation; μ=overall mean; R_i=fixed effect of replication (i=1, 2); W_j=fixed effect of weaning age (j=6 months, 8 months); S_k=fixed effect of sex (k=male, female); R_i x W_j=interaction between replication and weaning age; and e_{ijkl}=random residual. For ADG of the period between 6 and 8 months of age a model with all main effects and R_i x S_k=interaction between replication and sex, and e_{ijklm}=random residual, was used.

3.2 Trial 2 - Impact of weaning and castration on different traits in beef calves.

3.2.1 General

Trial 2 was executed at the experimental farm “Relliehausen” of the University of Goettingen, Lower Saxony, Germany, situated in the Solling uplands. The altitude of the farm was 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.

3.2.2 Animals, Treatments and Management

Over two consecutive years (2004 and 2005) a total of 64 male spring-born beef calves were examined. All animals were offspring of the farm-owned suckler cow herd. In each year at the end of the pasture season 32 male calves were randomly assigned to one of two treatments (16 calves/treatment):

1) weaning executed simultaneously with castration (WsC),
2) weaning occurred 4 weeks after castration (WaC).

Within each treatment there were a group of castrates (n=8) and a group of intact bulls (n=8), defined as control group.

All calves used in year 1 were offspring from crossbred cows (Limousin x German Holstein), each group included calves from one Limousin bull (LIM) and two Blonde d` Aquitaine bulls (BLA 1 and BLA 2). In year 2 purebred Aubrac calves (AUB; n=8) and offspring (n=24) from crossbred cows and BLA 1 and BLA 2, respectively, were investigated (Table 3.2).

Table 3.2 Trial 2 - Number of calves by breeding bull, treatment, sex and year

		Year 1				Year 2			
Treatment[1)]	Sex	LIM[2)]	BLA 1	BLA 2	Total	AUB	BLA 1	BLA 2	Total
WsC	Castrates	3	3	2	8	2	2	4	8
	Bulls	3	3	2	8	2	3	3	8
WaC	Castrates	3	3	2	8	2	3	3	8
	Bulls	3	3	2	8	2	3	3	8
Total		12	12	8	32	8	11	13	32

[1)] WsC = Weaning and castration occurred the same day. WaC = Weaning occurred 4 weeks after castration.
[2)] LIM = Limousin, BLA = Blonde d` Aquitaine, AUB = Aubrac.

One week before castration, all animals of the suckler cow herd were transferred from pasture into a solid cow stable. After weighing, the cow-calf pairs of the different treatment groups were allocated to one of four equal straw bedded compartments.

At the day of castration all calves were weighed and blood sampled. After castration WsC calves were separated into a solid weaning barn within shouting distance to their dams in the cow stable. So the weaned calves and their dams were able to have vocal communication with each other.

In the weaning barn the calves of both WsC groups were bedded on straw in similarly sized pens adjacent to each other (12.4 m^2/calf). There was a feed bunk in front of the pen, with 1.1 m/calf. The calves of the two different sex groups were able to have visible, tactile and olfactory contact to each other. All WaC calves remained with their dams in their respective compartments in the cow stable for a further 4-weeks period.

Four weeks after castration, the WaC calves were separated from their dams and placed into the weaning barn. They were housed in the same way as described for the WsC groups but without direct contact to them. After weaning the WaC calves, all calves of the trial were quartered in the weaning barn for a period of 3 weeks. After this 3-weeks period all calves left the weaning barn, they were regrouped within sex and moved into a further separate stable for the fattening period.

As long as the calves were housed with their dams, they had access to the feeding ration for the cows (straw and grass silage) The weaned calves were supplied with a feeding ration, consisting of grass silage (9.9 MJ ME/kg; 16.5% CP), but without any concentrates. One week before the fattening period started, the feeding ration of the calves were replaced stepwise by maize silage.

During fattening the animals got maize silage offered *ad libitum* and daily 2.5 kg of concentrates/animal. Concentrates consisted of field bean (65%), barley (32%) and minerals (3%).

Table 3.3 Trial 2 - Experimental procedures and measurements by treatment and time

	Treatment		
Code	WsC	WaC	Measurements
T-1[a)]	Housing in	Housing in	Body weight
T0	Weaning + Castration	Castration	Body weight, Blood sample
T1			Body weight, Blood sample
T2			Body weight, Blood sample
T3			Body weight, Blood sample
T4		Weaning	Body weight, Blood sample
T5			Body weight, Blood sample
T7	Start fattening period	Start fattening period	Body weight

[a)] T-1 = 1 week before T0. T1 = 1 week after T0.

3.2.3 Castration and Blood Sampling

The Burdizzo castration of the calves was performed in accordance to the German Animal Welfare law by an experienced veterinarian at T0. The animal was fixed in a crush. Additional ropes were used to hold its hind legs in a spread position to prevent injuries by movements of the animal. After injection of Xylazin (0.1 mg/kg BW) the castration was performed from behind through the legs of the calf. Each spermatic cord was crushed twice.

Table 3.4 Trial 2 - Age (d) (mean ± standard deviation) at castration by treatment, sex and year

Treatment[1)]	Sex	Year 1	Year 2
WsC	Castrates	197.3 ± 8.2	194.6 ± 13.4
	Bulls	192.0 ± 13.3	196.1 ± 16.0
WaC	Castrates	198.0 ± 15.7	193.4 ± 16.3
	Bulls	196.9 ± 10.8	193.5 ± 19.2

[1)] WsC = Weaning and castration occurred the same day. WaC = Weaning occurred 4 weeks after castration.

All animals were blood sampled by jugular venipuncture at T0 and weekly during a 5-weeks period after T0 (T1-T5) (Table 3.3). The values measured at T0 were used as pre-treatment baseline, therefore the T0 blood sample was taken before treatment procedures (castration and weaning) occurred. Similar, at T4 blood sampling was performed immediately before the weaning of the WaC treatment. Unclotted (EDTA) whole blood samples were analyzed for white blood cell counts (WBC) and the number and percentages of neutrophils and lymphocytes using a blood analyzer (Cell-Dyn 3500CS, Firma ABBOTT) at the Institute of Veterinary Medicine, University of Goettingen.

Blood samples for plasma Haptoglobin determination were collected into heparinized tubes and centrifuged at 3000 rpm at room temperature for 10 min at the laboratory of the Department of Animal Science, University of Goettingen. Plasma was subsequently stored at -20 °C until assayed for acute-phase protein Haptoglobin at the Institute of Animal Science, University of Bonn, as described by Orro et al. (2008).

3.2.4 Vocalization and Behavioral Observations

Direct observations using instantaneous scan sampling technique were applied in the same manner as described for Trial 1 to count the number of vocalizations coming from each group of calves after weaning. As modifications, in year 1 of Trial 2 there was a 10-min time period and the observation on d 1 started at 0900 and ended at 1800.

Types of behavior examined, sampling technique and sampling time table for behavioral observations were the same as described in Trial 1. However, in year 1 data were recorded using video monitoring instead of direct observations as in year 2.

3.2.5 Growth Performance

Body weight (BW), measured in kilogram (kg), of each calf was recorded 1 week before T0 (T-1), at castration (T0), weekly during the 5 weeks following T0 (T1-T5) and 7 weeks after T0 (T7). Average daily gain (ADG), measured in kilogram per day (kg/d), was calculated for a 1-week period before T0 (T-1 to T0), 3 weeks (T0 to T3) following T0, and 3 weeks (T4 to T7) after weaning of treatment WaC at T4.

3.2.6 Slaughter Traits

The animals were slaughtered on an individual basis, at a commercial abattoir, when they reached equitable, visually valuated body conformation and body weight relating to the actual market requirements. For each animal slaughter age, final BW, LWG, calculated from birth to slaughter, carcass weight and dressing percentage were recorded. Following slaughter, the carcasses were graded with regard to the degree of finishing (fat class) and the conformation class, into 1, 2, 3, 4, or 5, and EUROP categories, respectively. Both based on the European Union beef carcass classification system.

3.2.7 Statistical Analysis

All statistical analyses were conducted using the SAS program (Version 9.2; SAS Institute, 2008). All variables were analyzed using the GLIMMIX procedure to test for differences. Data are presented as least square means (lsmeans) with subscript standard errors (s.e.). Interactions between main effects were removed from the model when tested to be non-significant ($p \geq 0.05$). Multiple comparisons were done by applying the Tukey`s test (alpha=0.05).

For vocalization and behavior group of calves were served as experimental unit. Data were analyzed using a model with year (1 and 2), treatment (WsC and WaC), sex (castrates and bulls) and day after weaning (d 1, 2 and 3) as fixed main effects. Vocalization activity was analyzed considering the following model:

$$y_{ijklm}=\mu + A_i + T_j + S_k + D_l + A_i \times T_j + A_i \times S_k + T_j \times S_k + A_i \times T_j \times S_k + e_{ijklm},$$

where y_{ijklm}=the m^th^ observation; μ=overall mean; A_i =fixed effect of year (i= 1, 2); T_j=fixed effect of treatment (j=WsC, WaC); S_k=fixed effect of sex (k=castrates, bulls); D_l=fixed effect of day after weaning (l=1, 2, 3); A_i x T_j=interaction between year and treatment; A_i x S_k=interaction between year and sex; T_j x S_k=interaction between treatment and sex; A_i x T_j x S_k=three-way interaction between year, treatment and sex, and e_{ijklm}=random residual.

The model for feeding behavior included all main effects described before, and additional A_i x S_k=interaction between year and sex, A_i x T_j x S_k=three-way interaction between year, treatment and day after weaning, A_i x S_k x D_l=three-way interaction between year, sex and day after weaning, e_{ijklm}=random residual. Behavior types lying and standing/walking were both analyzed considering the model including all main effects and T_j x D_l=interaction between treatment and day after weaning, and e_{ijklm}=random residual.

Hematological parameters (Haptoglobin, WBC, number and percentage of neutrophils and lymphocytes), performance data (BW, ADG) and carcass traits were analyzed using individual calf records. The main effects of the model were year (1 and 2), treatment (WsC and WaC) and sex (castrates and bulls). The complete model was as follows:

$$y_{ijkl}=\mu + A_i + T_j + S_k + A_i \times T_j + A_i \times S_k + T_j \times S_k + A_i \times T_j \times S_k + e_{ijkl},$$

where y_{ijkl}=the l^{th} observation; μ=overall mean; A_i=fixed effect of year (i= 1, 2); T_j=fixed effect of treatment (j=WsC, WaC); S_k=fixed effect of sex (k=castrates, bulls); A_i x T_j=interaction between year and treatment; A_i x S_k=interaction between year and sex; T_j x S_k=interaction between treatment and sex; A_i x T_j x S_k=three-way interaction between year, treatment and sex, and e_{ijkl}=random residual.

3.3 Trial 3 - Effect of time of weaning on behavior and performance of crossbred beef calves after castration.

3.3.1 General

The site of Trial 3 was a commercial beef cattle farm in Oberweissbach, Thuringia, Germany. The farm is located in the high western part of the Thuringian Schiefergebirge (Slate Mountains), there are heights from 420 to 850 m above sea level, with 660 m in average. The farm used in total 1452 ha of agricultural area, including 1342 ha of grassland, with an east-west expansion of 43 km and a 10 km north-south extension. In this region rainfall is 842 mm annually on average, with 510 mm rainfall from April to October and a mean temperature of 5.9 °C (April-October: 10.7 °C).

3.3.2 Animals, Treatments and Management

Over two consecutive years a total of 111 crossbred male beef calves with origin from the farm were examined. In year 1 there were 66 calves (born from May 8 to August 31, 2004) included, and 45 calves (born from May 24 to August 25, 2005) were examined in year 2. Each year at the end of the pasture season the calves were randomly assigned to one of two treatments:

1) weaning occurred simultaneously with castration (WsC),
2) weaning was performed 8 weeks before castration (WbC).

The calves were offspring from 18 (year 1) and 23 (year 2) different Limousin bulls, respectively. Sired by the same 9 bulls were 63.6% (n=42) of the calves in year 1 and 64.7% of the calves (n=44) in year 2. The dams were predominately Simmental cows.

Table 3.5 Trial 3 - Number of calves by treatment, sex and year

Treatment	Sex	Year 1	Year 2	Total
WsC	Castrates	16	10	26
	Bulls	16	11	27
WbC	Castrates	17	12	29
	Bulls	17	12	29
Total		66	45	111

Table 3.6 Trial 3 - Experimental procedures and measurements by treatment

	Treatment		
Code	WsC	WbC	Measurements
T-8[a)]		Weaning	
T-5			
T0	Weaning + Castration	Castration	Body weight
T3[b)]			Body weight
TP[c)]			Body weight

[a)] 8 weeks before T0.
[b)] 3 weeks after T0. Beginning of fattening period for bulls and low energy input for castrates.
[c)] Beginning of pasture period.

3.3.3 Housing and Feeding Management

At least one week before weaning, the cow-calf pairs were housed in pens (up to 6 pairs/pen), to become acquainted to feeding stuff and facilities of the stable. Calves had free access to concentrates (6.7 MJ NEL; 18.0% CP) offered in troughs (1 trough/pen) and to water by self-watering drinking bowls (2 bowls/pen). The forage ration consisting of grass silage (10.0 MJ ME/kg DM; 14.4% CP) was delivered by an automatic feeding belt. The feeding belt was loaded up twice a day (between 0630 and 0730 as well as 1415 and 1445).

After separation, calves were placed into a stable adjacent to their dams` stable, thereby vocal communication between the weaned calves and their dams was made possible. The two calf groups of each treatment were allotted to adjacent equal-designed pens. Feeding regime for the weaned calves and housing facilities were the same as described before.

The pens (4.2 x 9.7 m) were constructed of metal gates and concrete walls, a concrete partly-slatted floor, a feeding and a lying area. The total lying area of a pen (around 40 m^2) was built by two rows of lying boxes (8 boxes/row) with solid concrete surface and a concrete partly-slatted pathway between the rows. The feeding area was defined as the area next to the feeder (feeding trough or feeding belt, respectively) with a rectangular shape, one side as long as the feeder and the other side as long as a calf's body length. There were 12 feeder sites along the feeding belt. The feeding trough was 2.0 m long and had a volume for approximately 230 kg of concentrates.

Up to 3 weeks after castration (4 weeks for WbC year 1) there was the same feeding regime for castrates and bulls. At the end of this period, the castrates of both treatments were mixed to a single herd of castrates.

Three weeks after castration (4 weeks for WbC bulls year 1), fattening of the bulls started, it was based on concentrates (6.7 MJ NEL; 18.0% CP; 7 kg/animal daily in the average of the complete fattening period) and grass silage (10.0 MJ ME; 14.4% CP) offered *ad libitum*. At the same time the extensive feeding period for the castrates began. This low energy input feeding ration (grass silage and straw) was supplied in preparation of the pasture period. Castrates started grazing on d 100 (WsC year 2), d 122 (WsC year 1) and d 128 (WbC both years) after castration. The pasture period in year 1 lasted 171 days; data from pasture period of year 2 were not recorded.

3.3.4 Castration

Because of the number of animals, there were two castration dates each year, according to the treatments. Average ages of the calves at castration are shown in Table 3.7. The Burdizzo castration procedure was performed in the same manner as described in Trial 2.

Table 3.7 Trial 3 - Age (d) (mean ± standard deviation) at castration by treatment, sex and year

Treatment[1)]	Sex	Year 1	Year 2
WsC	Castrates	204 ± 30	206 ± 27
	Bulls	210 ± 33	203 ± 28
WbC	Castrates	221 ± 21	216 ± 16
	Bulls	218 ± 19	214 ± 15

[1)] WsC = Weaning and castration occurred the same day. WbC = Weaning occurred 8 weeks before castration.

3.3.5 Vocalization and Behavioral Observations

Direct observations for vocalization activity and behavior of the calves on d 1, 2 and 3 after castration were conducted in the same manner as in Trial 1.

3.3.6 Growth Performance

Every calf`s body weight (BW), measured in kilogram (kg), was recorded at castration (T0), 3 weeks after castration (T3), and at the start of the pasture period (TP). The average daily gain (ADG), measured in kilogram per day (kg/d), was calculated for the 3-weeks period after castration (T0 to T3) and the pre-pasture period (T3 to TP).

3.3.7 Slaughter Traits

Animals were slaughtered at a commercial abattoir. Age at slaughter and carcass weight of each individual were recorded. Live weight gain (LWG), measured in kilogram per day (kg/d), was calculated from birth to slaughter.

3.3.8 Statistical Analysis

All statistical analyses were conducted using the SAS program (Version 9.2; SAS Institute, 2008). All variables were analyzed using the GLIMMIX procedure to test for differences. Data are presented as least square means (lsmeans) with subscript standard errors (s.e.). Interactions between main effects were removed from the model when tested to be non-significant ($p \geq 0.05$). Multiple comparisons were done by applying the Tukey`s test (alpha=0.05).

For analyzing vocalization and behavioral data group of calves served as an experimental unit. Vocalization after castration was analyzed using the following model:

$$y_{ijkl}=\mu + T_i + S_j + DC_k + T_i \times DC_k + e_{ijkl},$$

where y_{ijkl}=the l^{th} observation; μ=overall mean; T_i=fixed effect of treatment (i=WsC, WbC); S_j=fixed effect of sex (j=castrates, bulls); DC_k=fixed effect of day after castration (k=1, 2, 3); T_i x DC_k=interaction between treatment and day after castration, and e_{ijkl}=random residual. The model used for analyzing vocalization after castration + weaning was as followed:

$$y_{ijkl}=\mu + A_i + S_j + DCW_k + A_i \times S_j + A_i \times DCW_k + S_j \times DCW_k + A_i \times S_j \times DCW_k + e_{ijkl},$$

where y_{ijkl}=the l^{th} observation; μ=overall mean; A_i=fixed effect of year (i=1, 2); S_j=fixed effect of sex (j=castrates, bulls); DCW_k=fixed effect of day after castration + weaning (k=1, 2, 3); A_i x S_j=interaction between year and sex; A_i x DCW_k=interaction between year and day after castration + weaning; S_j x DCW_k=interaction between sex and day after castration + weaning; A_i x S_j x DCW_k=three-way interaction between year x sex x day after castration + weaning, and e_{ijkl}=random residual.

Feeding behavior was analyzed considering the following model:

$$y_{ijklm}=\mu + A_i + T_j + S_k + DC_l + T_j \times DC_l + e_{ijklm},$$

where y_{ijklm}=the m^{th} observation; μ=overall mean; A_i=fixed effect of year (i=1, 2), T_j=fixed effect of treatment (j=WsC, WbC); S_k=fixed effect of sex (k=castrates, bulls); DC_l=fixed effect of day after castration (l=1, 2, 3); T_j x DC_l=interaction between treatment and day after castration, and e_{ijklm}=random residual. For behavior type lying the same model as described for feeding behavior was used, but additionally including A_i x T_j=interaction between year and treatment; A_i x S_k=interaction between year and sex; and A_i x T_j x S_k=three-way interaction between year x treatment x sex. Standing/walking was analyzed considering the model described for behavior type lying, but without the three-way interaction.

For analyzing performance data and carcass traits, individual animal served as an experimental unit, the model included year (1 and 2), treatment (WsC and WbC), and sex (castrates and bulls), and the interactions between these main effects. The following model was used:

$$y_{ijkl}=\mu + A_i + T_j + S_k + A_i \times T_j + A_i \times S_k + T_j \times S_k + A_i \times T_j \times S_k + e_{ijkl},$$

where y_{ijkl}= the l^{th} observation; μ=overall mean; A_i=fixed effect of year (i=1, 2); T_j=fixed effect of treatment (j=WsC, WbC); S_k =fixed effect of sex (k=castrates, bulls); A_i x T_j=interaction between year and treatment; A_i x S_k=interaction between year and sex; T_j x S_k=interaction between treatment and sex; A_i x T_j x S_k=three-way interaction between year, treatment and sex, and e_{ijkl}=random residual.

4 RESULTS

4.1 Trial 1 - Influence of sex and age on behavior and performance in beef calves after weaning.

4.1.1 Trial 1 - Vocalization after Weaning

In replication 1 a lower (P=0.0489) vocalization activity was observed than in replication 2 (5.51 vs. 6.16 calls/10min; Table 4.1). W6 calves vocalized more (P<.0001) often than W8 calves (7.37 vs. 4.29 calls/10min), and female performed a higher (P=0.0008) number of vocalizations than male calves (6.39 vs. 5.27 calls/10min). The number of vocalizations was different between the days after weaning and decreased (P<.0001) from d 1 to d 3 (10.33, 5.43 and 1.73 calls/10min on d 1, 2 and 3, respectively; Table 4.1).

Table 4.1 Trial 1 - Vocalization of calves (calls/10min) (lsmeans $_{s.e.}$) after weaning by effects

Replication		Weaning age[1)]		Sex		Day after weaning		
1	2	W6	W8	Males	Females	1	2	3
$5.51^{a}_{\ 0.23}$	$6.16^{b}_{\ 0.23}$	$7.37^{a}_{\ 0.23}$	$4.29^{b}_{\ 0.23}$	$5.27^{a}_{\ 0.23}$	$6.39^{b}_{\ 0.23}$	$10.33^{a}_{\ 0.28}$	$5.43^{b}_{\ 0.28}$	$1.73^{c}_{\ 0.28}$

a, b, c = Values with different small letters are significantly different (p<0.05) within effect.
[1)] W6 = 6 months; W8 = 8 months.

A weaning age x day interaction (P=0.0002) and an interaction between sex and day (P=0.0048) was detected. Different levels of significance were found for the comparisons of W6 with W8 calves on d 1 (12.57 vs. 8.08 calls/10min; P<.0001), on d 2 (7.20 vs. 3.67 calls/10min; P<.0001), and on d 3 (2.35 vs. 1.12 calls/10min; P=0.0311; Table 4.2).

Table 4.2 Trial 1 - Vocalization of calves (calls/10min) (lsmeans $_{s.e.}$) on d 1, 2 and 3 after weaning of calves weaned at 6 or 8 months of age

Weaning age	Day after weaning		
	1	2	3
6 months	$12.57^{Aa}_{\ 0.40}$	$7.20^{Ab}_{\ 0.40}$	$2.35^{Ac}_{\ 0.40}$
8 months	$8.08^{Ba}_{\ 0.40}$	$3.67^{Bb}_{\ 0.40}$	$1.12^{Bc}_{\ 0.40}$

A, B = Column values with different capital letters are significantly different (p<0.05) between weaning ages (Tukey`s test).
a, b, c = Row values with different small letters are significantly different (p<0.05) between days (Tukey`s test).

On d 1 the female calves vocalized more often (P<.0001) than male calves (11.60 vs. 9.05 calls/10min). But there were no significant differences between the sexes found on d 2 (P=0.1219) and d 3 (P=0.9061) after weaning. (Table 4.3).

Table 4.3 Trial 1 - Vocalization of male and female calves (calls/10min) (lsmeans $_{s.e.}$) on d 1, 2 and 3 after weaning

Sex	Day after weaning		
	1	2	3
Male	$9.05^{Aa}_{\ 0.40}$	$5.00^{b}_{\ 0.40}$	$1.77^{c}_{\ 0.40}$
Female	$11.60^{Ba}_{\ 0.40}$	$5.87^{b}_{\ 0.40}$	$1.70^{c}_{\ 0.40}$

A, B = Column values with different capital letters are significantly different (p<0.05) between sexes (Tukey`s test).
a, b, c = Row values with different small letters are significantly different (p<0.05) between days (Tukey`s test).

4.1.2 Trial 1 - Behavior after Weaning

Feeding behavior was not influenced by year, treatment, sex or day after weaning in this trial. Lying and standing/walking of the calves was affected by weaning age and day after weaning, but not by replication and sex (Table 4.4). A higher percentage (P=0.026) of W8 than W6 calves was observed lying (43.8 vs. 39.1%). Conversely, more (P=0.001) W6 calves than W8 calves were observed standing/walking (44.7 vs. 39.1%; Table 4.4). The percentage of calves lying increased (P<.0001) from d 1 (22.3%) to d 2 (44.7%) and to d 3 (57.3%). Conversely, standing/walking was performed by more calves on d 1 than on d 2, and by more on d 2 compared with d 3 (61.3, 38.3 and 26.2% on d 1, 2 and 3, respectively; P<.0001; Table 4.4).

Table 4.4 Trial 1 - Behavior (% of calves) (lsmeans $_{s.e.}$) after weaning by effects

Effect	Class	Behavior		
		Feeding	Lying	Standing/walking
Replication	1	$15.6_{\ 0.83}$	$40.9_{\ 1.48}$	$43.5_{\ 1.22}$
	2	$17.7_{\ 0.83}$	$42.0_{\ 1.48}$	$40.3_{\ 1.22}$
Weaning age	6 months	$16.2_{\ 0.83}$	$39.1^{a}_{\ 1.48}$	$44.7^{a}_{\ 1.22}$
	8 months	$17.1_{\ 0.83}$	$43.8^{b}_{\ 1.48}$	$39.1^{b}_{\ 1.22}$
Sex	Male	$17.8_{\ 0.83}$	$41.2_{\ 1.48}$	$41.1_{\ 1.22}$
	Female	$15.6_{\ 0.83}$	$41.7_{\ 1.48}$	$42.7_{\ 1.22}$
Day after weaning	1	$16.4_{\ 1.02}$	$22.3^{a}_{\ 1.81}$	$61.3^{a}_{\ 1.49}$
	2	$17.1_{\ 1.02}$	$44.7^{b}_{\ 1.81}$	$38.3^{b}_{\ 1.49}$
	3	$16.5_{\ 1.02}$	$57.3^{c}_{\ 1.81}$	$26.2^{c}_{\ 1.49}$

a, b, c = Column values with different small letters are significantly different (p<0.05) within effect.

An interaction between replication and day affected lying (P=0.0068) and standing/walking (P=0.0054) was observed. The percentage of calves lying, differed (P=0.0246) between replication 1 and replication 2 only on d 1 (26.4 vs. 18.3%), but not on d 2 (P=0.1682) and d 3 (P=0.0663; Table 4.5). For standing/walking (P=0.1187) no difference between replication 1 and 2 was detected on d 1. On d 2 more (P=0.0343) calves were observed standing/walking in replication 1 compared to replication 2 (41.4 vs. 35.1%; Table 4.5). Similarly, on d 3 more (P=0.0082) calves were standing/walking in replication 1 than in replication 2 (30.2 vs. 22.3%; Table 4.5). Within both replications, all days after weaning were different from each other for the behavior types lying and standing/walking, details are displayed by Table 4.5.

Table 4.5 Trial 1 - Lying and standing/walking (% of calves) (lsmeans $_{s.e.}$) on d 1, 2 and 3 after weaning by replication

Behavior	Replication	Day after weaning		
		1	2	3
Lying	1	26.4^{Aa} $_{2.56}$	42.2^{b} $_{2.56}$	54.0^{c} $_{2.56}$
	2	18.3^{Ba} $_{2.56}$	47.2^{b} $_{2.56}$	60.7^{c} $_{2.56}$
Standing/walking	1	58.9^{a} $_{2.11}$	41.4^{Ab} $_{2.11}$	30.2^{Ac} $_{2.11}$
	2	63.6^{a} $_{2.11}$	35.1^{Bb} $_{2.11}$	22.3^{Bc} $_{2.11}$

A, B = Column values with different capital letters are significantly different (p≤0.05) between replications (Tukey`s test).
a, b, c = Row values with different small letters are significantly different (p<0.05) between days (Tukey`s test).

A weaning age x day interaction influenced lying (P<.0001) and standing/walking (P=0.0016). On d 2 less (P<.0001) W6 than W8 calves were lying (36.3 vs. 53.1%), and conversely, more (P<.0001) W6 than W8 calves were observed standing/walking (45.4 vs. 31.1%; Table 4.6). However, on d 1 and d 3 differences between the weaning ages were not detected. Within each weaning age the lying and standing/walking data were different between d 1 and d 2, between d 2 and d 3 and between d 1 and d 3 (Table 4.6).

Table 4.6 Trial 1 - Lying and standing/walking (% of calves) (lsmeans $_{s.e.}$) after weaning by weaning age and day after weaning

Behavior	Weaning age	Day after weaning		
		1	2	3
Lying	6 months	24.1^{a} $_{2.56}$	36.3^{Ab} $_{2.56}$	57.0^{c} $_{2.56}$
	8 months	20.6^{a} $_{2.56}$	53.1^{Bb} $_{2.56}$	57.7^{b} $_{2.56}$
Standing/walking	6 months	61.3^{a} $_{2.11}$	45.4^{Ab} $_{2.11}$	27.4^{c} $_{2.11}$
	8 months	61.2^{a} $_{2.11}$	31.1^{Bb} $_{2.11}$	25.0^{c} $_{2.11}$

A, B = Column values with different capital letters are significantly different (p≤0.05) between weaning ages (Tukey`s test).
a, b, c = Row values with different small letters are significantly different (p<0.05) between days (Tukey`s test).

4.1.3 Trial 1 - Growth Performance after Weaning

Replication did not affect performance of the calves in this trial (Table 4.7). One week before weaning W6 calves were almost 60 kg lighter (P<.0001) compared to W8 calves (240.9 vs. 299.2 kg), and male calves were more than 20 kg heavier (P=0.0021) than female calves (280.8 vs. 259.3 kg; Table 4.7). W8 had a greater (P<.0001) weaning weight compared with the W6 calves (300.6 vs. 249.3 kg), and the female were lighter (P=0.0015) at weaning than the male calves (264.1 vs. 285.7 kg; Table 4.7). Furthermore, the BW at weaning was affected by a replication x weaning age interaction (P=0.0244). The weaning weight differed (P=0.0310) for the W8 calves between replication 1 and 2 (310.8 vs. 290.4 kg), but not for the W6 calves (P=0.2968; Table 4.8).

Table 4.7 Trial 1 - Age (d), body weight (kg), live weight gain (kg/d) and average daily gain (kg/d) (lsmeans $_{s.e.}$) of the calves at different times by effects

Item	Replication		Weaning age		Sex	
	1	2	6 months	8 months	Male	Female
Age, d						
at weaning	216.0 $_{0.82}$	217.6 $_{0.82}$	192.4^{a} $_{0.82}$	241.3^{b} $_{0.82}$	217.2 $_{0.82}$	216.4 $_{0.82}$
Body weight, kg						
1 week before weaning	270.8 $_{4.76}$	269.3 $_{4.76}$	240.9^{a} $_{4.76}$	299.2^{b} $_{4.76}$	280.8^{a} $_{4.76}$	259.3^{b} $_{4.76}$
at weaning	277.6 $_{4.64}$	272.3 $_{4.64}$	249.3^{a} $_{4.64}$	300.6^{b} $_{4.64}$	285.7^{a} $_{4.64}$	264.1^{b} $_{4.64}$
2 weeks after weaning	285.2 $_{4.63}$	283.7 $_{4.63}$	258.2^{a} $_{4.63}$	310.8^{b} $_{4.63}$	298.8^{a} $_{4.63}$	270.2^{b} $_{4.63}$
at 6 months of age	254.6 $_{4.17}$	251.7 $_{4.17}$	249.3 $_{4.17}$	257.0 $_{4.17}$	261.7^{a} $_{4.17}$	244.6^{b} $_{4.17}$
at 8 months of age	286.3 $_{4.71}$	282.5 $_{4.71}$	268.3^{a} $_{4.71}$	300.6^{b} $_{4.71}$	296.4^{a} $_{4.71}$	272.4^{b} $_{4.71}$
Live weight gain, kg/d						
birth to 6 months of age	1.11 $_{0.02}$	1.09 $_{0.02}$	1.08 $_{0.02}$	1.12 $_{0.02}$	1.14^{a} $_{0.02}$	1.06^{b} $_{0.02}$
birth to 8 months of age	1.01 $_{0.02}$	1.00 $_{0.02}$	0.94^{a} $_{0.02}$	1.07^{b} $_{0.02}$	1.05^{a} $_{0.02}$	0.96^{b} $_{0.02}$
Average daily gain, kg/d						
1 week before weaning	0.98 $_{0.18}$	0.49 $_{0.18}$	1.29^{a} $_{0.18}$	0.18^{b} $_{0.18}$	0.74 $_{0.18}$	0.73 $_{0.18}$
2 weeks after weaning	0.59 $_{0.08}$	0.79 $_{0.08}$	0.61 $_{0.08}$	0.76 $_{0.08}$	0.94^{a} $_{0.08}$	0.43^{b} $_{0.08}$
6 to 8 months of age[1)]	0.65 $_{0.04}$	0.63 $_{0.04}$	0.39^{a} $_{0.04}$	0.89^{b} $_{0.04}$	0.71^{a} $_{0.04}$	0.57^{b} $_{0.04}$

a, b = Row values with different small letters are significantly different (p<0.05) within effect.
[1)] period of 7 weeks between the weaning dates of the two weaning ages.

The BW measured 2 weeks after weaning was influenced by weaning age (P<.0001) and sex (P<.0001; Table 4.7), and an interaction between replication and weaning age (P=0.0045) was detected (Table 4.8). Two weeks after weaning, males were heavier (P<.0001) than females (298.8 vs. 270.2 kg). W6 calves were lighter (P<.0001) than W8 (258.2 vs. 310.8 kg; Table 4.7).

For the W8 the BW measured 2 weeks after weaning was greater (P=0.029) in replication 1 compared to replication 2 (321.2 vs. 300.5 kg). In contrast, there was no significant difference (P=0.0600) between the replications detected for W6 (Table 4.8).

Live weight gain (LWG) calculated from birth to 6 months of age was influenced only by sex (P=0.0140) and an interaction between replication and weaning age (P=0.0286). Male calves had a greater LWG to 6 months of age than female calves (1.14 vs. 1.06 kg/d; Table 4.7). In replication 1 the W6 calves had smaller (P=0.0137) LWG to 6 months of age than the W8 calves (1.06 vs. 1.16 kg/d), but in replication 2 no difference between weaning ages was found (Table 4.8). LWG to 6 months of age of W8 calves was greater (P=0.0492) in replication 1 than replication 2 (1.16 vs. 1.08 kg/d), but there was no difference (P=0.2504) detected between the replications for W6 calves (Table 4.8).

LWG, calculated from birth to 8 months of age, was greater (P<.0001) for the W8 calves compared to W6 calves (1.07 vs. 0.94 kg/d) and greater (P=0.0013) for the male than female calves (1.05 vs. 0.96 kg/d; Table 4.7). Furthermore, there was an interaction between replication and weaning age found for this LWG. Within replication 1 there was a smaller LWG to 8 months of age (P<.0001) for the W6 than W8 calves (0.95 vs. 1.11 kg/d; Table 4.8), whereas no difference between the weaning ages was detected in replication 2.

During 1 week before weaning the W6 calves had a greater (P<.0001) ADG than the W8 calves (1.29 vs. 0.18 kg/d; Table 4.7). Sex did not affect ADG during 1 week before weaning. An interaction between replication and weaning age was detected (P=0.0334). ADG during 1 week before weaning did not significantly differ (P=0.1283) between the weaning ages in replication 1, though there was a biological difference detectable with a greater ADG for W6 compared to W8 calves (1.26 vs. 0.70 kg/d; Table 4.8). However, in replication 2 ADG was greater (P<.0001) for W6 than for W8 calves (1.33 vs. –0.34 kg/d; Table 4.7). Additionally, for the W8 calves there was a difference (P=0.0054) detected between the replications, but not for the W6 calves (P=0.8431; Table 4.8).

ADG, calculated for 2 weeks after weaning, was not influenced by weaning age, but male calves had a greater (P<.0001) ADG than female calves (0.94 vs. 0.43 kg/d; Table 4.7). Furthermore, a replication x weaning age interaction was found. In replication 1 a greater (P=0.0084) ADG was calculated for W8 compared to W6 calves (0.80 vs. 0.38 kg/d), but in replication 2 there was no difference (P=0.4032) between the weaning ages (Table 4.8).

Table 4.8 Trial 1 - Age (d), body weight (kg), live weight gain (kg/d) and average daily gain (kg/d) (lsmeans $_{s.e.}$) of the calves at different times by weaning age and replication

Item	Weaning age	Replication 1	Replication 2
Body weight, kg			
at weaning	6 months	244.4^{A} $_{6.56}$	254.2^{A} $_{6.56}$
	8 months	310.8^{Ba} $_{6.56}$	290.4^{Bb} $_{6.56}$
2 weeks after weaning[2)]	6 months	249.3^{A} $_{6.55}$	267.0^{A} $_{6.55}$
	8 months	321.2^{Ba} $_{6.55}$	300.5^{Bb} $_{6.55}$
6 months of age	6 months	244.4^{A} $_{5.90}$	254.2 $_{5.90}$
	8 months	264.9^{B} $_{5.90}$	249.2 $_{5.90}$
8 months of age	6 months	261.9 $_{6.66}$	274.7 $_{6.66}$
	8 months	310.8 $_{6.66}$	290.4 $_{6.66}$
Live weight gain, kg/d			
birth to 6 months of age	6 months	1.06^{A} $_{0.03}$	1.10 $_{0.03}$
	8 months	1.16^{Ba} $_{0.03}$	1.08^{b} $_{0.03}$
birth to 8 months of age	6 months	0.95^{A} $_{0.03}$	0.97 $_{0.03}$
	8 months	1.11^{B} $_{0.03}$	1.03 $_{0.03}$
Average daily gain, kg/d			
1 week before weaning	6 months	1.26 $_{0.26}$	1.33^{A} $_{0.26}$
	8 months	0.70^{a} $_{0.26}$	-0.34^{Bb} $_{0.26}$
2 weeks after weaning	6 months	0.38^{Aa} $_{0.11}$	0.85^{b} $_{0.11}$
	8 months	0.80^{B} $_{0.11}$	0.72 $_{0.11}$

A, B = Column values with different capital letters are significantly different (p<0.05) between weaning ages (Tukey`s test).
a, b = Row values with different small letters are significantly different (p<0.05) between replications (Tukey`s test).

The BW at 6 months of age was influenced by sex, the male calves were heavier (P=0.0048), than the female calves (261.7 vs. 244.6 kg; Table 4.7). Additionally, an interaction between replication and weaning age (P=0.0346) was detected for the 6-months BW. There was no difference (P=0.5550) between the weaning ages detected in replication 2, but in replication 1 the W8 calves had a greater (P=0.0166) 6-months BW than the W6 calves (264.9 vs. 244.4 kg; Table 4.8).

The body weight at 8 months of age was affected by weaning age and sex. The 8-months BW of the W8 calves was more than 30 kg higher (P<.0001) than that of the W6 (300.6 vs. 268.3 kg; Table 4.7). Bulls were at 8 months of age almost 25 kg heavier (P=0.0006) than same-age females (296.4 vs. 272.4 kg; Table 4.7). For the 8-months BW a replication x weaning age interaction was detected (Table 4.8).

The growth rate during the period between the weaning dates of the two weaning ages (ADG 6 to 8 months) was influenced by weaning age (P<.0001) and sex (P=0.0196), and a replication x sex interaction (P=0.0021) was found. W6 had a lower (P<.0001) ADG compared to W8 calves (0.39 vs. 0.89 kg/d), and male had a greater (P=0.0196) ADG than the female calves (0.71 vs. 0.57 kg/d; Table 4.7). However, in replication 1 the male calves had a greater (P=0.0002) ADG than the female calves (0.81 vs. 0.48 kg/d), whereas in replication 2 no difference (P= 0.5720) between the sexes was detected (Table 4.9).

Table 4.9 Trial 1 - Average daily gain (kg/d) (lsmeans $_{s.e.}$) of male and female calves by replication

	Sex	Replication 1	Replication 2
Average daily gain, kg/d			
6 to 8 months of age	Male	$0.81^{Aa}\ _{0.06}$	$0.60^{b}\ _{0.06}$
	Female	$0.48^{Ba}\ _{0.06}$	$0.65^{b}\ _{0.06}$

A, B = Column values with different capital letters are significantly different (p≤0.05) between sexes (Tukey`s test).
a, b = Row values with different small letters are significantly different (p<0.05) between replications (Tukey`s test).

4.2 Trial 2 - Impact of weaning and castration on different traits in beef calves.

4.2.1 Trial 2 - Vocalization after Weaning

In year 1 a higher (P<.0001) vocalization activity was detected than in year 2 (2.71 vs. 1.77 calls/10min). WsC calves shouted more often (P<.0001) than WaC calves (3.20 vs. 1.28 calls/10min) and castrates vocalized less (P=0.0002) than bulls (1.84 vs. 2.64 calls/10min; Table 4.10). The number of vocalizations decreased from d 1 to d 3 after weaning and was different (P<.0001) between the days (4.69, 1.59 and 0.44 calls/10min on d 1, 2 and 3, respectively; Table 4.10).

Table 4.10 Trial 2 - Vocalization of calves (calls/10min) (lsmeans $_{s.e.}$) after weaning by effects

Year		Treatment[1)]		Sex		Day after weaning		
1	2	WsC	WaC	Castrates	Bulls	1	2	3
$2.71^{a}_{0.15}$	$1.77^{b}_{0.15}$	$3.20^{a}_{0.15}$	$1.28^{b}_{0.15}$	$1.84^{a}_{0.15}$	$2.64^{b}_{0.15}$	$4.69^{a}_{0.19}$	$1.59^{b}_{0.19}$	$0.44^{c}_{0.19}$

a, b, c = Values with different small letters are significantly different (p<0.05) within effect (Tukey`s test).
[1)] WsC = Weaning and castration occurred the same day. WaC = Weaning occurred 4 weeks after castration.

Interactions between year and treatment (P<.0001) as well as year and sex (P<.0001) were found. In both years, WsC calves shouted more often than WaC calves (Table 4.11). The number of vocalizations of the WsC calves was different (P<.0001) between the years (4.18 and 2.22 calls/10min in year 1 and 2, respectively), but not the vocalization of the WaC calves.

Table 4.11 Trial 2 - Vocalization of calves (calls/10min) (lsmeans $_{s.e.}$) after weaning, when weaned and castrated the same day or weaned 4 weeks after castration by year

Treatment[1)]	Year 1	Year 2
WsC	$4.18^{Aa}_{0.22}$	$2.22^{Ab}_{0.22}$
WaC	$1.24^{B}_{0.22}$	$1.32^{B}_{0.22}$

A, B = Column values with different capital letters are significantly different (p<0.05) between treatments (Tukey`s test).
a, b = Row values with different small letters are significantly different (p<0.05) between years (Tukey`s test).
[1)] WsC = Weaning and castration occurred the same day. WaC = Weaning occurred 4 weeks after castration.

In year 1 bulls vocalized (P<.0001) more than twice as much compared to castrates (3.63 vs. 1.79 calls/10 min), but in year 2 no difference between the sexes was detected (Table 4.12). There was no difference found in terms of vocalization activity of the castrates between the years. However, more (P<.0001) bulls vocalized in year 1 than year 2 (3.63 vs. 1.66 calls/10 min; Table 4.12).

Table 4.12 Trial 2 - Vocalization of castrates and bulls (calls/10min) (lsmeans $_{s.e.}$) after weaning by year

Sex	Year 1	Year 2
Castrates	1.79^{A} $_{0.22}$	1.89 $_{0.22}$
Bulls	3.63^{Ba} $_{0.22}$	1.66^{b} $_{0.22}$

A, B = Column values with different capital letters are significantly different (p<0.05) between sexes (Tukey`s test).
a, b = Row values with different small letters are significantly different (p<0.05) between years (Tukey`s test).
1) WsC = Weaning and castration occurred the same day. WaC = Weaning occurred 4 weeks after castration.

A treatment x sex interaction (P=0.0009) was detected and an interaction between treatment and day after weaning (P<.0001). WsC bulls shouted more often (P<.0001) than WsC castrates (3.97 vs. 2.43 calls/10min), but within WaC treatment no difference (P=0.7997) was found between the sexes (Table 4.13). The WsC castrates shouted more often (P=0.0001) than the WaC castrates (2.43 vs. 1.24 calls/10min) and the WsC bulls vocalized more often (P<.0001) than the WaC bulls (3.97 vs. 1.32 calls/10min; Table 4.13).

Table 4.13 Trial 2 - Vocalization of castrates and bulls (calls/10min) (lsmeans $_{s.e.}$) on d 1, 2 and 3 after weaning, when weaned and castrated the same day or weaned 4 weeks after castration

Sex	Treatment[1)]	
	WsC	WaC
Castrates	2.43^{Aa} $_{0.22}$	1.24^{b} $_{0.22}$
Bulls	3.97^{Ba} $_{0.22}$	1.32^{b} $_{0.22}$

A, B = Column values with different capital letters are significantly different (p<0.05) between sexes (Tukey`s test).
a, b = Row values with different small letters are significantly different (p<0.05) between treatments (Tukey`s test).
1) WsC = Weaning and castration occurred the same day. WaC = Weaning occurred 4 weeks after castration.

There was difference in vocalization between the treatments on d 1 (P<.0001) and d 2 (P<.0001) but not on d 3 (P=0.1435; Table 4.14). An interaction year x treatment x sex (P<.0001) was detected to have an influence on vocalization activity in this trial.

Table 4.14 Trial 2 - Vocalization of calves (calls/10min) (lsmeans $_{s.e.}$) on d 1, 2 and 3 after weaning, when weaned and castrated the same day or weaned 4 weeks after castration

Treatment[1)]	Day after weaning		
	1	2	3
WsC	6.43^{Aa} $_{0.27}$	2.45^{Ab} $_{0.27}$	0.72^{c} $_{0.27}$
WaC	2.95^{Ba} $_{0.27}$	0.73^{Bb} $_{0.27}$	0.17^{b} $_{0.27}$

A, B, C = Column values with different capital letters are significantly different (p<0.05) between treatments (Tukey`s test).
a, b, c = Row values with different small letters are significantly different (p<0.05) between days (Tukey`s test).
1) WsC = Weaning and castration occurred the same day. WaC = Weaning occurred 4 weeks after castration.

4.2.2 Trial 2 - Behavior after Weaning

Sex did not affect any of the observed types of behavior. Feeding was not influenced by treatment, while year did not affect lying and standing/walking.

In year 1 less (P=0.0071) calves were observed feeding than in year 2 (16.1 vs. 20.0%). More WsC calves (P=0.0002) were lying compared to WaC calves (48.0 vs. 38.8%), and a lower (P<.0001) percentage of WsC calves than WaC calves was observed standing/walking (33.7 vs. 43.4%; Table 4.15). All types of behavior were influenced by day after weaning (P<.0001). Most calves (23.9%) were observed feeding on d 3 compared to d 1 (17.2%) and d 2 (13.1%; Table 4.15). On d 1 less calves (32.7%) were lying than on d 2 (50.3%) and d 3 (47.1%). The percentage of calves observed standing/walking decreased from d 1 to d 3 after weaning (Table 4.15).

Table 4.15 Trial 2 - Behavior (% of calves) (lsmeans $_{s.e.}$) by effects

Effect	Class	Behavior		
		Feeding	Lying	Standing/walking
Year	1	$16.1^{a}_{1.0}$	$44.8_{1.8}$	$39.1_{1.4}$
	2	$20.0^{b}_{1.0}$	$42.0_{1.8}$	$38.1_{1.4}$
Treatment[1)]	WsC	$18.3_{1.0}$	$48.0^{a}_{1.8}$	$33.7^{a}_{1.4}$
	WaC	$17.8_{1.0}$	$38.8^{b}_{1.8}$	$43.4^{b}_{1.4}$
Sex	Castrates	$18.0_{1.0}$	$42.2_{1.8}$	$39.8_{1.4}$
	Bulls	$18.1_{1.0}$	$44.5_{1.8}$	$37.4_{1.4}$
Day after weaning	1	$17.2^{a}_{1.2}$	$32.7^{a}_{2.2}$	$50.1^{a}_{1.7}$
	2	$13.1^{b}_{1.2}$	$50.3^{b}_{2.2}$	$36.6^{b}_{1.7}$
	3	$23.9^{c}_{1.2}$	$47.1^{b}_{2.2}$	$29.0^{c}_{1.7}$

a, b, c = Column values with different small letters are significantly different (p<0.05) within effect.
[1)] WsC = Weaning and castration occurred the same day. WaC = Weaning occurred 4 weeks after castration.

For feeding a year x sex interaction (P=0.0110) was detected. In year 1 less (P=0.0002) bulls were observed feeding than in year 2 (14.4 vs. 21.8%), but there was no difference (P=0.9171) for castrates between the years (17.9 and 18.1% for year 1 and 2, respectively; Table 4.16). There were no significant differences between castrates and bulls detected within any one year (Table 4.16).

Furthermore, the treatment x sex x day after weaning three-way interaction (P=0.0354) was found to affect the behavior type feeding in this trial.

Table 4.16 Trial 2 - Feeding behavior of castrates and bulls (% of calves) (lsmeans $_{s.e.}$) after weaning by year

Behavior	Sex	Year 1	Year 2
Feeding	Castrates	17.9 $_{1.4}$	18.1 $_{1.4}$
	Bulls	14.4^{a} $_{1.4}$	21.8^{b} $_{1.4}$

A, B = Column values with different capital letters are significantly different (p<0.05) between sexes (Tukey`s test).
a, b = Row values with different small letters are significantly different (p<0.05) between years (Tukey`s test).

An interaction between treatment and day after weaning was detected for lying (P<.0001) and standing/walking (P=0.0002). On d 1 there were differences found between the treatments for lying (P<.0001) and standing/walking (P<.0001), but not on d 2 and d 3 (Table 4.17).

Table 4.17 Trial 2 - Lying and standing/walking (% of calves) (lsmeans $_{s.e.}$) on d 1, 2 and 3 after weaning, when weaned and castrated the same day or weaned 4 weeks after castration

Behavior	Treatment[1)]	Day after weaning		
		1	2	3
Lying	WsC	44.7^{Aa} $_{3.0}$	53.3^{b} $_{3.0}$	45.9^{ab} $_{3.0}$
	WaC	20.7^{Ba} $_{3.0}$	47.3^{b} $_{3.0}$	48.3^{b} $_{3.0}$
Standing/walking	WsC	39.6^{Aa} $_{2.4}$	33.5^{b} $_{2.4}$	28.0^{b} $_{2.4}$
	WaC	60.5^{Ba} $_{2.4}$	39.7^{b} $_{2.4}$	30.0^{c} $_{2.4}$

A, B = Column values with different capital letters are significantly different (p<0.05) between treatments (Tukey`s test).
a, b, c = Row values with different small letters are significantly different (p<0.05) between days (Tukey`s test).
[1)] WsC = Weaning and castration occurred the same day. WaC = Weaning occurred 4 weeks after castration.

4.2.3 Trial 2 - Blood Traits

The concentration of acute-phase protein Haptoglobin in the serum of the calves` blood was not affected by sex at any of the sampling times. The pre-treatment baseline value (T0) for Haptoglobin concentration was more than twice as much (P=0.002) in year 2 compared to year 1 (744.3 vs. 297.5 µg/ml) and greater (P=0.0476) for WaC compared to WsC calves (660.9 vs. 380.9 µg/ml; Table 4.18). Haptoglobin concentration at T1, T2 and T3 was not influenced by year, treatment or sex. The effect year influenced Haptoglobin at T4 and T5, on both sampling times there were greater values detected in year 1 compared to year 2 (Table 4.18).

The total white blood cell count (WBC) were not influenced by any of the effects at T0, T1 and T4. Castrates had a greater amount of WBC than bulls at T2 (P=0.0301), T3 (P=0.0458) and at T5 (P=0.0078), details are shown in Table 4.18.

At T0 the number of neutrophils was not influenced by any of the effects or interactions between them. It was also not affected by sex at any sampling time (T0 to T5). At T1 there were more (P=0.0254) neutrophils in year 1 than in year 2 (2.16 vs. 1.53 K/µL; Table 4.18). At T2 and T3 the neutrophil number was not influenced by year, treatment or sex. However, there was a year x sex interaction found at T2 (P=0.0303) and T3 (P=0.0378; Table 4.20) and an additional interaction between year and treatment at T3 (P=0.0012; Table 4.19). At T4 the number of neutrophils was higher (P=0.0036) for WaC compared to WsC calves (1.44 vs. 0.94 K/µL; Table 4.18), and a year x treatment interaction (P=0.0036) was detected. At T4 in year 1 WsC had less (P<.0001) neutrophils than WaC calves, but no significant treatment difference was found for year 2 (Table 4.19). Furthermore, at T4 the WsC calves had a higher number (P=0.0095) of neutrophils in year 2 compared to year 1 (1.25 vs. 0.63 K/µL). The opposite was found for WaC calves with more (P=0.0360) neutrophils in year 1 than in year 2 (1.69 vs. 1.19 K/µL; Table 4.19). At T5 there were less (P=0.0001) neutrophils in year 1 than in year 2 (0.89 vs. 1.44 K/µL; Table 4.18), and more (P=0.0001) neutrophils found for WaC compared to WsC calves (1.44 vs. 0.89 K/µL; Table 4.18). Furthermore, there was a noticeable interaction between year and treatment at this sampling time. WaC had more (P<.0001) neutrophils than WsC (1.50 vs. 0.27 K/µL) in year 1, but no difference between the treatments was found in year 2 (Table 4.19). At T5 the number of neutrophils for WsC calves was higher (P<.0001) in year 2 compared to year 1 (1.50 vs. 0.27 K/µL), but did not differ for WaC between the years (Table 4.19).

The percentage of neutrophils at T0 was significantly influenced by a year x treatment interaction (P=0.0325), but not by the effects. In year 1 at T0 there was a higher percentage of neutrophils for WaC calves than WsC calves (22.63 vs. 16.51 K/ µL), and conversely in year 2, a higher percentage of neutrophils for WsC compared to WaC calves was found (24.38 vs. 17.89 K/ µL), but no significant difference between the treatments was detected (Table 4.19).

Year influenced the percentage of neutrophils at T1, showing a higher (P=0.0012) value in year 1 than year 2 (27.19 vs. 18.03%; Table 4.18). At T2 and T3, none of the effects influenced the percentage of neutrophils, however, at T2 a year x sex interaction and at T3 a year x treatment interaction was observed. At T2 there was no difference between the sexes in year 1, but in year 2 the bulls had a higher (P=0.0155) percentage of neutrophils than castrates (25.63 vs. 18.44%; Table 4.20). At this sampling time the castrates in year 1 had a higher (P=0.0227) percentage of neutrophils compared to year 2 (25.19 vs. 18.44%). For the bulls no difference between the years was found (Table 4.20).

Table 4.18 Trial 2 - Blood traits of calves (lsmeans $_{s.e.}$) on different sampling times by effects

Blood traits	Year		Treatment[1)]		Sex	
Sampling time[2)]	1	2	WsC	WaC	Castrates	Bulls
Haptoglobin, µg/ml						
T0	$297.5^{a}_{97.9}$	$744.3^{b}_{97.9}$	$380.9^{a}_{97.9}$	$660.9^{b}_{97.9}$	$544.8_{97.9}$	$497.0_{97.9}$
T1	$528.5_{154.8}$	$585.9_{157.4}$	$499.7_{157.4}$	$614.7_{154.8}$	$707.6_{157.4}$	$406.8_{154.8}$
T2	$236.5_{140.8}$	$469.0_{140.8}$	$252.0_{140.8}$	$453.5_{140.8}$	$251.6_{140.8}$	$453.9_{140.8}$
T3	$301.9_{129.0}$	$306.5_{129.0}$	$195.8_{129.0}$	$412.5_{129.0}$	$250.3_{129.0}$	$358.1_{129.0}$
T4	$326.1^{a}_{80.2}$	$90.6^{b}_{80.2}$	$142.3_{80.2}$	$274.4_{80.2}$	$129.3_{80.2}$	$287.4_{80.2}$
T5	$409.2^{a}_{56.8}$	$114.2^{b}_{55.8}$	$248.5_{56.8}$	$274.8_{55.8}$	$233.3_{55.8}$	$290.1_{56.8}$
WBC, K/µL						
T0	$8.66_{0.47}$	$8.47_{0.47}$	$8.41_{0.47}$	$8.72_{0.47}$	$8.84_{0.47}$	$8.29_{0.47}$
T1	$8.19_{0.43}$	$8.09_{0.43}$	$8.50_{0.43}$	$7.78_{0.43}$	$8.47_{0.43}$	$7.81_{0.43}$
T2	$8.16_{0.40}$	$7.97_{0.40}$	$8.00_{0.40}$	$8.13_{0.40}$	$8.69^{a}_{0.40}$	$7.44^{b}_{0.40}$
T3	$7.75_{0.36}$	$8.09_{0.36}$	$8.00_{0.36}$	$7.84_{0.36}$	$8.44^{a}_{0.36}$	$7.41^{b}_{0.36}$
T4	$7.13_{0.36}$	$8.03_{0.36}$	$7.28_{0.36}$	$7.88_{0.36}$	$7.91_{0.36}$	$7.25_{0.36}$
T5	$6.73_{0.31}$	$7.38_{0.31}$	$7.13_{0.31}$	$6.97_{0.31}$	$7.66^{a}_{0.31}$	$6.44^{b}_{0.31}$
Neutrophils, K/µL						
T0	$1.61_{0.15}$	$1.81_{0.15}$	$1.64_{0.15}$	$1.78_{0.15}$	$1.81_{0.15}$	$1.61_{0.15}$
T1	$2.16^{a}_{0.19}$	$1.53^{b}_{0.19}$	$1.75_{0.19}$	$1.94_{0.19}$	$1.91_{0.19}$	$1.78_{0.19}$
T2	$1.88_{0.12}$	$1.75_{0.12}$	$1.84_{0.12}$	$1.78_{0.12}$	$1.88_{0.12}$	$1.75_{0.12}$
T3	$1.53_{0.10}$	$1.59_{0.10}$	$1.53_{0.10}$	$1.59_{0.10}$	$1.56_{0.10}$	$1.56_{0.10}$
T4	$1.16_{0.12}$	$1.22_{0.12}$	$0.94^{a}_{0.12}$	$1.44^{b}_{0.12}$	$1.06_{0.12}$	$1.31_{0.12}$
T5	$0.89^{a}_{0.10}$	$1.44^{b}_{0.09}$	$0.89^{a}_{0.10}$	$1.44^{b}_{0.09}$	$1.09_{0.09}$	$1.23_{0.10}$
Neutrophils, %						
T0	$19.57_{2.03}$	$21.13_{2.03}$	$20.44_{2.03}$	$20.26_{2.03}$	$20.66_{2.03}$	$20.04_{2.03}$
T1	$27.19^{a}_{1.90}$	$18.03^{b}_{1.90}$	$20.84_{1.90}$	$24.38_{1.90}$	$22.56_{1.90}$	$22.66_{1.90}$
T2	$24.25_{1.44}$	$22.03_{1.44}$	$24.00_{1.44}$	$22.28_{1.44}$	$21.81_{1.44}$	$24.47_{1.44}$
T3	$21.03_{1.56}$	$20.50_{1.56}$	$19.81_{1.56}$	$21.72_{1.56}$	$18.84_{1.56}$	$22.69_{1.56}$
T4	$16.34_{1.40}$	$15.28_{1.40}$	$12.16^{a}_{1.40}$	$19.47^{b}_{1.40}$	$13.91_{1.40}$	$17.72_{1.40}$
T5	$16.18^{a}_{1.37}$	$20.41^{b}_{1.34}$	$13.99^{a}_{1.37}$	$22.59^{b}_{1.34}$	$16.47_{1.34}$	$20.12_{1.37}$
Lymphocytes, K/µL						
T0	$6.22_{0.45}$	$6.11_{0.45}$	$6.03_{0.45}$	$6.29_{0.45}$	$6.29_{0.45}$	$6.03_{0.45}$
T1	$5.19_{0.40}$	$5.81_{0.40}$	$6.03_{0.40}$	$4.97_{0.40}$	$5.81_{0.40}$	$5.19_{0.40}$
T2	$5.50_{0.35}$	$5.63_{0.35}$	$5.53_{0.35}$	$5.59_{0.35}$	$6.22^{a}_{0.35}$	$4.91^{b}_{0.35}$
T3	$5.53_{0.37}$	$5.91_{0.37}$	$5.88_{0.37}$	$5.56_{0.37}$	$6.28^{a}_{0.37}$	$5.16^{b}_{0.37}$
T4	$5.38_{0.34}$	$6.16_{0.34}$	$5.75_{0.34}$	$5.78_{0.34}$	$6.28^{a}_{0.34}$	$5.25^{b}_{0.34}$
T5	$4.91_{0.31}$	$5.31_{0.30}$	$5.44_{0.31}$	$4.78_{0.30}$	$5.66^{a}_{0.30}$	$4.57^{b}_{0.31}$
Lymphocytes, %						
T0	$70.19_{2.39}$	$68.44_{2.39}$	$69.38_{2.39}$	$69.26_{2.39}$	$68.32_{2.39}$	$70.31_{2.39}$
T1	$61.91^{a}_{2.35}$	$71.56^{b}_{2.35}$	$69.44_{2.35}$	$64.03_{2.35}$	$67.31_{2.35}$	$66.16_{2.35}$
T2	$66.56_{1.80}$	$69.06_{1.80}$	$67.25_{1.80}$	$68.38_{1.80}$	$70.19_{1.80}$	$65.44_{1.80}$
T3	$70.00_{2.05}$	$70.22_{2.05}$	$71.66_{2.05}$	$68.56_{2.05}$	$72.16_{2.05}$	$68.06_{2.05}$
T4	$74.28_{1.65}$	$75.25_{1.65}$	$78.84^{a}_{1.65}$	$70.69^{b}_{1.65}$	$77.41^{a}_{1.65}$	$72.13^{b}_{1.65}$
T5	$74.10_{1.84}$	$70.50_{1.84}$	$76.94^{a}_{1.84}$	$67.66^{b}_{1.84}$	$74.66_{1.84}$	$69.94_{1.84}$

a, b = Row values with different small letters are significantly different ($p<0.05$) with effect (Tukey`s test).
[1)] WsC = Weaning and castration occurred the same day. WaC = Weaning occurred after castration.
[2)] T0 = castration and weaning of WsC. Blood sampling before castration / weaning occurred. T1 = 1 week after weaning WsC. T4 = Weaning of WaC. Blood sampling before weaning occurred.

At T3 the WsC had a lower (P=0.0367) percentage of neutrophils than WaC calves in year 1 (17.69 vs. 24.38%), but no difference between treatments was found in year 2 (Table 4.19).

At T4 the WsC calves had a lower (P=0.0005) percentage of neutrophils than WaC calves (12.16 vs. 19.47%; Table 4.18) and an interaction between year and treatment was found. There was no difference between the treatments in year 2. However, in year 1 WsC had a lower (P=0.0367) percentage of neutrophils compared to WaC calves (9.19 vs. 23.50%; Table 4.19). Furthermore, at T4 the WsC calves had higher (P=0.0378) percentage of neutrophils in year 2 than year 1 (15.13 vs. 9.19%). The opposite was found for WaC calves having a higher (P=0.0054) percentage of neutrophils in year 1 compared to year 2 (23.50 vs. 15.44%; Table 4.19).

Table 4.19 Trial 2 - Blood traits of calves (lsmeans $_{s.e.}$) at different sampling times, when weaned and castrated the same day or weaned 4 weeks after castration by year

Blood traits / Sampling time[2)]	Treatment[1)]	Year 1	Year 2
Neutrophils, K/µL			
T3	WsC	$1.25^{Aa}_{0.15}$	$1.81^{Ab}_{0.15}$
	WaC	$1.81^{Ba}_{0.15}$	$1.38^{Bb}_{0.15}$
T4	WsC	$0.63^{Aa}_{0.16}$	$1.25^{b}_{0.16}$
	WaC	$1.69^{Ba}_{0.16}$	$1.19^{b}_{0.16}$
T5	WsC	$0.27^{Aa}_{0.14}$	$1.50^{b}_{0.14}$
	WaC	$1.50^{B}_{0.14}$	$1.38_{0.14}$
Neutrophils, %			
T0	WsC	$16.51_{2.92}$	$24.38_{2.92}$
	WaC	$22.63_{2.92}$	$17.89_{2.92}$
T3	WsC	$17.69^{A}_{2.21}$	$21.94_{2.21}$
	WaC	$24.38^{B}_{2.21}$	$19.06_{2.21}$
T4	WsC	$9.19^{Aa}_{1.98}$	$15.13^{b}_{1.98}$
	WaC	$23.50^{Ba}_{1.98}$	$15.44^{b}_{1.98}$
T5	WsC	$6.04^{Aa}_{1.96}$	$21.94^{b}_{1.90}$
	WaC	$26.31^{Ba}_{1.90}$	$18.88^{b}_{1.90}$
Lymphocytes, K/µL			
T5	WsC	$5.70^{A}_{0.44}$	$5.19_{0.44}$
	WaC	$4.13^{Ba}_{0.44}$	$5.44^{b}_{0.44}$
Lymphocytes, %			
T0	WsC	$74.07_{3.44}$	$64.69_{3.44}$
	WaC	$66.31_{3.44}$	$72.20_{3.44}$
T4	WsC	$82.06^{A}_{2.34}$	$75.63_{2.34}$
	WaC	$66.50^{Ba}_{2.34}$	$74.88^{b}_{2.34}$
T5	WsC	$85.44^{Aa}_{2.64}$	$68.44^{b}_{2.56}$
	WaC	$62.75^{Ba}_{2.56}$	$72.56^{b}_{2.56}$

A, B = Column values with different capital letters are significantly different (p<0.05) between treatments (Tukey`s test).
a, b = Row values with different small letters are significantly different (p<0.05) between years (Tukey`s test).
[1)] WsC = Weaning and castration occurred the same day. WaC = Weaning occurred after castration.
[2)] T0 = castration and weaning of WsC. Blood sampling before castration / weaning occurred. T1 = 1 week after weaning WsC. T4 = Weaning of WaC. Blood sampling before weaning occurred.

The percentage of neutrophils at T5 was influenced by year and treatment, and an interaction between these effects. In year 1 there was a lower (P=0.0315) percentage of neutrophils compared to year 2 (16.18 vs. 20.41%) and WaC calves had a higher (P<.0001) percentage of neutrophils than WsC calves (22.59 vs. 13.99%; Table 4.18). At T5 in year 1 the WaC had a higher (P<.0001) percentage of neutrophils compared to WsC calves (26.31 vs. 6.04%). In year 2, however, treatments were not different from each other (Table 4.19). At this sampling time WsC calves had a lower (P<.0001) percentage of neutrophils in year 1 than in year 2 (6.04 vs. 21.94%), but conversely the WaC had a higher (P=0.0076) percentage of neutrophils in year 1 compared to year 2 (26.31 vs. 18.88%; Table 4.19).

Table 4.20 Trial 2 - Blood traits of castrates and bulls (lsmeans $_{s.e.}$) at different sampling times by year

Blood traits / Sampling time[1)]	Sex	Year 1	Year 2
Neutrophils, K/µL			
T2	Castrates	2.13^{Aa} $_{0.17}$	1.63^{b} $_{0.17}$
	Bulls	1.63^{B} $_{0.17}$	1.88 $_{0.17}$
T3	Castrates	1.69 $_{0.15}$	1.44 $_{0.15}$
	Bulls	1.38 $_{0.15}$	1.75 $_{0.15}$
Neutrophils, %			
T2	Castrates	25.19^{a} $_{2.04}$	18.44^{Ab} $_{2.04}$
	Bulls	23.31 $_{2.04}$	25.63^{B} $_{2.04}$
Lymphocytes; %			
T2	Castrates	66.19^{a} $_{2.54}$	74.19^{Ab} $_{2.54}$
	Bulls	66.94 $_{2.54}$	63.94^{B} $_{2.54}$

A, B = Column values with different capital letters are significantly different (p<0.05) between sexes (Tukey`s test).
a, b = Row values with different small letters are significantly different (p<0.05) between years (Tukey`s test).
[1)] T0 = castration and weaning of WsC. Blood sampling before castration / weaning occurred. T1 = 1 week after weaning WsC. T4 = Weaning of WaC. Blood sampling before weaning occurred.

The number of lymphocytes at T0 and T1 was not influenced by any of the effects or interactions between them. The effects year and treatment did not affect the number of lymphocytes at T2 to T5. Castrates had a higher number of lymphocytes compared to bulls at T2 (P=0.01), T3 (P=0.0370), T4 (P=0.0347) and T5 (P=0.0145), details are shown in Table 4.18. At T5 additionally an influence of the year x treatment interaction (P=0.0393) was found. At T5 the number of lymphocytes in year 1 was smaller (P=0.0134) for WaC compared to WsC calves (4.13 vs. 5.70 K/µL), but treatments did not differ in year 2 (Table 4.19). There was no difference between the years at T5 for WsC calves, but WaC calves had a higher (P=0.0344) number of lymphocytes in year 2 than year 1 (5.44 vs. 4.13 K/µL; Table 4.19).

At T0 there was no effect of year, treatment or sex on the percentage of lymphocytes detected. However, at T0 this hematological parameter was influenced by a year x treatment interaction (P=0.0278), but no significant differences within years or treatments were detected (Table 4.19). At T1 a lower (P=0.0052) percentage of lymphocytes was found in year 1 than in year

1 (61.91 vs. 71.56%; Table 4.18). At T2 and T3 there was no influence of any of the effects or interactions on percentage of lymphocytes. At T4 percentage of lymphocytes was higher (P=0.0009) for WsC compared to WaC calves (78.84 vs. 70.69%) and higher (P=0.0275) for castrates than bulls (77.41 vs. 72.13%; Table 4.18). Furthermore, an interaction between year and treatment was detected at T4 (P=0.0024), that showed in year 1 a higher (P<.0001) percentage of lymphocytes for WsC compared to WaC calves (82.06 vs. 66.50%; Table 4.19). The percentage of lymphocytes for WaC calves at T4 was lower (P=0.0140) in year 1 compared to year 2 (66.50 vs. 74.88%), but for WsC calves no difference between the years was detected (Table 4.19). At T5 the WsC calves had a higher (P=0.0007) percentage of lymphocytes than WaC calves (76.94 vs. 67.66%; Table 4.18). Additionally, at this sampling time the percentage of lymphocytes was influenced by a year x treatment interaction (P<.0001). In year 1, WsC had higher (P<.0001) percentage of lymphocytes than WaC calves (85.44 vs. 62.75%). Conversely, in year 2 there was a higher percentage of lymphocytes for WaC calves compared to WsC calves (72.56 vs. 68.44%; Table 4.19). At T5 in year 1, WsC had a higher (P<.0001) percentage of lymphocytes than WaC calves (85.44 vs. 62.75%). No difference was detected between the treatments for year 2 (Table 4.19).

4.2.4 Trial 2 – Growth Performance

BW of the calves before and during the 7-weeks period following T0 and LWG, calculated from birth to T0 and T4, were not influenced year, treatment or sex (Table 4.21).

During the last week before T0 (T-1 to T0) the WsC calves showed greater (P<.0001) weight loss than the WaC calves (-0.82 vs. -0.37 kg/d; Table 4.21). Year and sex did not influence the ADG during this period (Table 4.21). An effect of the three-way-interaction year x treatment x sex was detected (P=0.0362).

ADG during the 3 weeks following T0 (T0 to T3) was affected by year and treatment. In year 1, a smaller (P<.0001) ADG than in year 2 was found (0.49 vs. 1.13 kg/d). The WsC calves had higher (P=0.0151) ADG than the WaC calves (0.94 vs. 0.68 kg/d) in this period (Table 4.21). ADG from T4 to T7 (3 weeks after weaning WaC) was higher (P<.0001) for WaC compared to WsC calves (0.62 vs. 0.10 kg/d) but not influenced by year and sex (Table 4.21).

Table 4.21 Trial 2 - Age (d), body weight (kg), live weight gain (kg/d), and average daily gain (kg/d) (lsmeans $_{s.e.}$) of the calves at different times by effects

Traits Time	Year 1	 2	Treatment[2)] WsC	 WaC	Sex Castrates	 Bulls
Age, d						
T0	196.0 $_{2.5}$	194.4 $_{2.5}$	195.0 $_{2.5}$	195.4 $_{2.5}$	195.8 $_{2.5}$	194.6 $_{2.5}$
at weaning[3)]	210.0 $_{2.5}$	208.4 $_{2.5}$	195.0 $_{2.5}$	223.4 $_{2.5}$	209.8 $_{2.5}$	208.6 $_{2.5}$
Body weight, kg						
T-1	260.4 $_{6.1}$	255.3 $_{6.1}$	258.9 $_{6.1}$	256.8 $_{6.1}$	253.9 $_{6.1}$	261.8 $_{6.1}$
T0	257.0 $_{6.0}$	249.3 $_{6.0}$	252.3 $_{6.0}$	253.9 $_{6.0}$	249.7 $_{6.0}$	256.5 $_{6.0}$
T3	267.2 $_{6.6}$	273.0 $_{6.6}$	272.0 $_{6.6}$	268.2 $_{6.6}$	265.6 $_{6.6}$	274.6 $_{6.6}$
T4	267.1 $_{6.5}$	277.5 $_{6.5}$	272.2 $_{6.5}$	272.4 $_{6.5}$	268.7 $_{6.5}$	275.9 $_{6.5}$
T7	276.3 $_{6.3}$	283.3 $_{6.3}$	274.3 $_{6.3}$	285.3 $_{6.3}$	275.6 $_{6.3}$	284.1 $_{6.3}$
at weaning	262.3 $_{6.3}$	262.5 $_{6.3}$	252.3^{a} $_{6.3}$	272.4^{b} $_{6.3}$	258.6 $_{6.3}$	266.1 $_{6.3}$
Live weight gain, kg/d						
birth to T0	1.06 $_{0.02}$	1.03 $_{0.02}$	1.04 $_{0.02}$	1.06 $_{0.02}$	1.03 $_{0.02}$	1.06 $_{0.02}$
birth to T4	0.98 $_{0.02}$	1.03 $_{0.02}$	1.00 $_{0.02}$	1.01 $_{0.02}$	1.00 $_{0.02}$	1.02 $_{0.02}$
Average daily gain, kg/d						
T-1 to T0[4)]	-0.43 $_{0.15}$	-0.76 $_{0.15}$	-0.82^{a} $_{0.15}$	-0.37^{b} $_{0.15}$	-0.53 $_{0.15}$	-0.66 $_{0.15}$
T0 to T3	0.49^{a} $_{0.07}$	1.13^{b} $_{0.07}$	0.94^{a} $_{0.07}$	0.68^{b} $_{0.07}$	0.76 $_{0.07}$	0.86 $_{0.07}$
T4 to T7	0.44 $_{0.06}$	0.28 $_{0.06}$	0.10^{a} $_{0.06}$	0.62^{b} $_{0.06}$	0.33 $_{0.06}$	0.39 $_{0.06}$

a, b = Row values with different small letters are significantly different (p<0.05) within effect.
[1)] T0 = Castration and weaning of WsC treatment.
[2)] WsC = Weaning and castration occurred the same day. WaC = Weaning occurred 4 weeks after castration.
[3)] Weaning of WsC treatment occurred at castration = T0. Weaning of WA treatment occurred after castration = T4.
[4)] T-1 to T0 = 1 week before T0. T0 to T3 = 3 weeks after T0. T4 to T7 = 3 weeks after T4.

4.2.5 Trial 2 - Slaughter Traits

There was no treatment effect on any of the slaughter traits analyzed in this trial (Table 4.22). Slaughter age was influenced by year (P=0.0079) and sex (P=0.0009) as well as year x sex interaction (P<.0001). In year 1, the animals were in average more than 2 weeks older (P=0.0079) compared to year 2 when they were slaughtered (512.7 vs. 495.9 days). Castrates were in average more than 3 weeks younger (P=0.0009) than bulls (493.6 vs. 515.0 days; Table 4.22). In year 2, castrates were slaughtered at a younger age (P<.0001) compared to bulls (472.3 vs. 519.6 days). However, there was no difference between the sexes in year 1 (Table 4.23). The slaughter age of the castrates was lower (P<.0001) in year 2 than year 1 (472.3 vs. 515.0 days) but did not differ (P=0.2860) for the bulls (Table 4.23).

The final BW was affected by sex and a year x sex interaction. BW of the bulls at slaughter was almost 90 kg higher (P<.0001) compared to the castrates (651.1 vs. 563.6 kg; Table 4.22). In year 2, the bulls were heavier (P=0.0026) at slaughter than in year 1 (684.2 vs. 617.9 kg) but there was no difference (P=0.4603) between the years for the final BW of the castrates (571.3 and 555.9 kg for year 1 and 2, respectively; Table 4.23). In each year, the final BW of the castrates was lower than that of the bulls (Table 4.23). LWG, calculated from birth to slaughter, was not influenced by treatment. In year 1, animals had a smaller (P=0.0019) LWG compared to year 2 (1.07 vs. 1.15 kg/d) and bulls had a greater (P<.0001) LWG than castrates (1.17 vs. 1.05 kg/d; Table 4.22).

Table 4.22 Trial 2 - Different slaughter traits (lsmeans $_{s.e.}$) by effects

Traits	Year		Treatment[1)]		Sex	
	1	2	WsC	WaC	Castrates	Bulls
Slaughter age, d	512.7^{a} $_{4.3}$	495.9^{b} $_{4.3}$	501.8 $_{4.3}$	506.9 $_{4.3}$	493.6^{a} $_{4.3}$	515.0^{b} $_{4.3}$
Final body weight, kg	594.6 $_{10.3}$	620.0 $_{10.5}$	604.9 $_{10.3}$	609.7 $_{10.5}$	563.6^{a} $_{10.3}$	651.1^{b} $_{10.5}$
Live weight gain; kg/d birth to slaughter	1.07^{a} $_{0.02}$	1.15^{b} $_{0.02}$	1.11 $_{0.02}$	1.11 $_{0.02}$	1.05^{a} $_{0.02}$	1.17^{b} $_{0.02}$
Carcass weight, kg	350.3 $_{6.8}$	360.4 $_{6.9}$	355.7 $_{6.8}$	355.0 $_{6.9}$	321.2^{a} $_{6.8}$	389.5^{b} $_{6.9}$
Dressing, %	58.9 $_{0.5}$	57.9 $_{0.5}$	58.7 $_{0.5}$	58.1 $_{0.5}$	57.0^{a} $_{0.5}$	59.8^{b} $_{0.5}$
Conformation class[2)]	2.94^{a} $_{0.07}$	2.57^{b} $_{0.08}$	2.75 $_{0.07}$	2.76 $_{0.08}$	3.03^{a} $_{0.07}$	2.48^{b} $_{0.08}$
Fat class[3)]	2.8^{a} $_{0.1}$	2.4^{b} $_{0.1}$	2.6 $_{0.1}$	2.6 $_{0.1}$	2.9^{a} $_{0.1}$	2.3^{b} $_{0.1}$

a, b = Row values with different small letters are significantly different (p<0.05) within effect.
[1)] WsC = Weaning and castration occurred the same day. WaC = Weaning occurred 4 weeks after castration.
[2)] Carcass conformation classes by EUROP classification scale: E=1=worst; U=2; R=3, O=4; P=5=best.
[3)] Fat classes by EUROP classification scale: 1= leanest to 5 = fattest.

Sex (P<.0001) and an interaction between year and sex (P=0.0060) influenced carcass weight. The castrates had lighter (P<.0001) carcasses than the bulls (321.2 vs. 389.5 kg; Table 4.22). In year 2, the bulls had heavier (P=0.0083) carcass weights than in year 1 (408.3 vs. 370.7 kg) but carcasses of the castrates did not differ (P=0.2027) between the years (Table 4.23).

For the bulls a higher (P<.0001) dressing percentage was found compared to the castrates (59.8 vs. 57.0%; Table 4.22). The classification of the carcasses for conformation (EUROP) and fat class (1 to 5) was affected by year and sex. In year 2, the carcasses were classified in a better (P=0.0011) EUROP and in a lower (P=0.0102) fat class compared to year 1 (Table 4.22). The carcasses of the castrates reached a conformation class, characterizing lower (P<.0001) conformation, compared to the bull carcasses (3.03 vs. 2.48). The castrates` carcasses had a higher (P=0.0002) fat class than the bull`s carcasses (2.9 vs. 2.3; Table 4.22).

Table 4.23 Trial 2 - Different slaughter traits (lsmeans $_{s.e.}$) of castrates and bulls by year

Traits	Sex	Year 1	Year 2
Slaughter age, d	Castrates	515.0^{a} $_{6.0}$	472.3^{Ab} $_{6.0}$
	Bulls	510.3 $_{6.0}$	519.6^{B} $_{6.2}$
Final body weight, kg	Castrates	571.3^{A} $_{14.6}$	555.9^{A} $_{14.6}$
	Bulls	617.9^{Ba} $_{14.6}$	684.2^{Bb} $_{15.1}$
Carcass weight, kg	Castrates	329.9^{A} $_{9.6}$	312.4^{A} $_{9.6}$
	Bulls	370.7^{Ba} $_{9.6}$	408.3^{Bb} $_{9.9}$

A, B = Column values with different capital letters are significantly different (p<0.05) between sexes (Tukey`s test).
a, b = Row values with different small letters are significantly different (p<0.05) between years (Tukey`s test).

4.3 Trial 3 - Effect of time of weaning on behavior and performance of crossbred beef calves after castration.

4.3.1 Trial 3 - Vocalization after Castration

Analysis of vocalization activity after castration including both treatments was done using observation data from year 2 only. Treatment (P<.0001) and day after castration (P<.0001) affecting the number of vocalizations. There was less (P<.0001) vocalization activity after castration of WbC compared to WsC calves (0.01 vs. 1.12 calls/10min). The number of vocalizations decreased (P<.0001) from d 1 to d 3 after castration (Table 4.24).

Table 4.24 Trial 3 - Vocalization of calves (calls/10min) (lsmeans $_{s.e.}$) after castration by effects

Treatment[1)]		Sex		Day after castration		
WsC	WbC	Castrates	Bulls	1	2	3
$1.12^{a}_{0.08}$	$0.01^{b}_{0.08}$	$0.59_{0.08}$	$0.54_{0.08}$	$1.18^{a}_{0.09}$	$0.42^{b}_{0.09}$	$0.10^{c}_{0.09}$

a, b, c = Values with different small letters are significantly different (p<0.05) within effect.
[1)] WsC = Weaning and castration occurred the same day. WbC = Weaning occurred 8 weeks before castration.

A treatment x day after castration interaction was detected (P<.0001) showing that the WsC calves vocalized more (P<.0001) than the WbC calves on d 1 (2.35 vs. 0.01 calls/10min) and d 2 (0.82 vs. 0.01 calls/10min; P<.0001) after castration (Table 4.25). However, on d 3 after castration there was no difference (P=0.2816) found between the treatments (Table 4.25).

Table 4.25 Trial 3 - Vocalization of calves (calls/10min) (lsmeans $_{s.e.}$) on d 1, 2 and 3 after castration, when weaned and castrated the same day or weaned 8 weeks before castration

Treatment[1)]	Day after castration		
	1	2	3
WsC	$2.35^{Aa}_{0.13}$	$0.82^{Ab}_{0.13}$	$0.20^{c}_{0.13}$
WbC	$0.01^{B}_{0.13}$	$0.01^{B}_{0.13}$	$0.00_{0.13}$

A, B = Column values with different capital letters are significantly different (p≤0.05) between treatments (Tukey`s test).
a, b, c = Row values with different small letters are significantly different (p<0.05) between days (Tukey`s test).
[1)] WsC = Weaning and castration occurred the same day. WbC = Weaning occurred 8 weeks before castration.

4.3.2 Trial 3 - Vocalization after Castration + Weaning

A second analysis was made including vocalization data from treatment WsC of both years, recorded on d 1, 2 and 3 after castration, which occurred the same day as weaning for this treatment.

The effects year, sex and day after castration + weaning influenced the number of vocalizations after castration + weaning. In year 1, calves were vocalizing more (P<.0001) than twice as often as calves in year 2 (2.60 vs. 1.12 calls/10 min). Bulls were observed to vocalize more (P=0.0071) than castrates (2.08 vs. 1.64 calls/10min; Table 4.26). The number of vocalizations decreased (P<.0001) in the course of the days after castration + weaning from d 1 (3.34 calls/10min) to d 2 (1.75 calls/10min) and to d 3 (0.49 calls/10min; Table 4.26).

Table 4.26 Trial 3 - Vocalization of WsC calves (calls/10min) (lsmeans $_{s.e.}$) after castration + weaning by effects

Year		Sex		Day after castration + weaning		
1	2	Castrates	Bulls	1	2	3
$2.60^{a}_{0.11}$	$1.12^{b}_{0.11}$	$1.64^{a}_{0.11}$	$2.08^{b}_{0.11}$	$3.34^{a}_{0.14}$	$1.75^{b}_{0.14}$	$0.49^{c}_{0.14}$

a, b, c = Values with different small letters are significantly different (p<0.05) within effect.

There was a year x sex interaction (P=0.0009), showing more (P<.0001) vocalizations from bulls than castrates in year 1 (3.10 vs. 2.10 calls/10min). However, no difference (P=0.6370) between bulls and castrates was found in year 2 (Table 4.27). For both sexes the number of vocalizations was greater in year 1 than year 2, details are displayed in Table 4.27.

Table 4.27 Trial 3 - Vocalization of WsC castrates and bulls (calls/10min) (lsmeans $_{s.e.}$) after castration + weaning by year

Sex	Year 1	Year 2
Castrates	$2.10^{Aa}_{0.16}$	$1.18^{b}_{0.16}$
Bulls	$3.10^{Ba}_{0.16}$	$1.07^{b}_{0.16}$

A, B = Column values with different capital letters are significantly different (p≤0.05) between years (Tukey`s test).
a, b = Row values with different small letters are significantly different (p<0.05) between sexes (Tukey`s test).

There was an interaction found between year and day after castration + weaning (P=0.0006) as well as sex and day after castration + weaning (P=0.0170). In year 1 higher vocalization activity was observed than in year 2 on d 1 (4.34 vs. 2.35 calls/10min; P<.0001), on d 2 (2.68 vs. 0.82 calls/10min; P<.0001) and on d 3 (0.77 vs. 0.20 calls/10min; P=0.0425; Table 4.28).

Table 4.28 Trial 3 - Vocalization of WsC calves (calls/10min) (lsmeans $_{s.e.}$) on d 1, 2 and 3 after castration + weaning by year

Year	Day after castration + weaning		
	1	2	3
1	4.34^{Aa} $_{0.20}$	2.68^{Ab} $_{0.20}$	0.77^{Ac} $_{0.20}$
2	2.35^{Ba} $_{0.20}$	0.82^{Bb} $_{0.20}$	0.20^{Bc} $_{0.20}$

A, B = Column values with different capital letters are significantly different (p≤0.05) between years (Tukey`s test).
a, b, c = Row values with different small letters are significantly different (p<0.05) between days (Tukey`s test).

On d 2, bulls vocalized more (P<.0001) than castrates (2.30 vs. 1.20 calls/10min) but no differences between the sexes were found on d 1 (P=0.4225) and d 3 (P=0.9792; Table 4.29). A three-way interaction including year, sex and day after castration + weaning was detected for vocalization considering analyzed WsC data.

Table 4.29 Trial 3 - Vocalization of WsC castrates and bulls (calls/10min) (lsmeans $_{s.e.}$) on d 1, 2 and 3 after castration + weaning

Sex	Day after castration + weaning		
	1	2	3
Castrates	3.23^{a} $_{0.20}$	1.20^{Ab} $_{0.20}$	0.48^{c} $_{0.20}$
Bulls	3.46^{a} $_{0.20}$	2.30^{Bb} $_{0.20}$	0.49^{c} $_{0.20}$

A, B = Column values with different capital letters are significantly different (p≤0.05) between sexes (Tukey`s test).
a, b, c = Row values with different small letters are significantly different (p<0.05) between days (Tukey`s test).

4.3.3 Trial 3 - Behavior after Castration

Feeding behavior after castration was not influenced by treatment or sex. In year 1 more (P=0.0027) calves were observed feeding than in year 2 (24.2 vs. 20.0%; Table 4.30). Percentage of calves observed feeding increased (P<.0001) from d 1 to d 3 after castration. On d 2 there were more calves (P=0.0431) feeding than on d 1 (21.9 vs. 18.5%) and less (P<0.0001) calves compared to d 3 (25.9 vs. 21.9%; Table 4.30). An interaction between treatment and day after castration for feeding behavior was detected with a difference (P=0.0133) between the treatments only on d 3 after castration (22.9 and 28.9% for WbC and WsC, respectively; Table 4.33).

More bulls (P<.0001) were observed lying than castrates (29.1 vs. 15.0%; Table 4.30) while more (P<.0001) castrates than bulls were standing/walking (62.6 vs. 49.2%). A lower percentage (P=0.0463) of WsC calves was observed standing/walking than WbC calves (54.3 vs. 57.4%; Table 4.30). Behavior type standing/walking was affected by day after castration (P<.0001). On d 1 more (P=0.0010) calves were standing/walking than on d 2 (61.4 vs. 54.1%) and also more (P<.0001) compared to d 3 (61.4 vs. 52.1%; Table 4.30). There was no significant difference (P=0.2761) between d 2 and d 3 for standing/walking.

Table 4.30 Trial 3 - Behavior (% of calves) (lsmeans $_{s.e.}$) after castration by effects

Effect	Class	Behavior		
		Feeding	Lying	Standing/walking
Year	1	24.2^{a} $_{1.0}$	20.9 $_{1.3}$	54.9 $_{1.1}$
	2	20.0^{b} $_{1.0}$	23.1 $_{1.3}$	56.8 $_{1.1}$
Treatment[1)]	WsC	22.6 $_{1.0}$	23.1 $_{1.3}$	54.3^{a} $_{1.1}$
	WbC	21.6 $_{1.0}$	21.0 $_{1.3}$	57.4^{b} $_{1.1}$
Sex	Castrates	22.5 $_{1.0}$	15.0^{a} $_{1.3}$	62.6^{a} $_{1.1}$
	Bulls	21.7 $_{1.0}$	29.1^{b} $_{1.3}$	49.2^{b} $_{1.1}$
Day after weaning	1	18.5^{a} $_{1.2}$	20.1 $_{1.5}$	61.4^{a} $_{1.3}$
	2	21.9^{b} $_{1.2}$	24.0 $_{1.5}$	54.1^{b} $_{1.3}$
	3	25.9^{c} $_{1.2}$	22.0 $_{1.5}$	52.1^{b} $_{1.3}$

a, b, c = Column values with different small letters are significantly different (p<0.05) within effect.
[1)] WsC = Weaning and castration occurred the same day. WbC = Weaning occurred 8 weeks before castration.

An interaction between year and treatment (P=0.0011) and a year x sex interaction (P=0.0009) for lying was detected. In year 2 less (P=0.0018) WbC than WsC calves were lying (19.2 vs. 27.1%; Table 4.31). Lying behavior of WsC calves differed (P=0.0014) between the years (19.0 vs. 27.1% in year 1 and 2, respectively; Table 4.31).

Table 4.31 Trial 3 - Behavior (% of calves) (lsmeans $_{s.e.}$) after castration, when weaned and castrated the same day or weaned 8 weeks before castration by year

Behavior	Treatment[1)]	Year 1	Year 2
Lying	WsC	19.0^{a} $_{1.8}$	27.1^{Ab} $_{1.8}$
	WbC	22.8 $_{1.8}$	19.2^{B} $_{1.8}$
Standing/walking	WsC	56.5^{a} $_{1.5}$	52.2^{Ab} $_{1.5}$
	WbC	53.3^{a} $_{1.5}$	61.5^{Bb} $_{1.5}$

A, B = Column values with different capital letters are significantly different (p<0.05) between treatments (Tukey`s test).
a, b = Row values with different small letters are significantly different (p<0.05) between years (Tukey`s test).
[1)] WsC = Weaning and castration occurred the same day. WbC = Weaning occurred 8 weeks before castration.

In year 1 (P=0.0011) and year 2 (P<.0001) more bulls than castrates were observed lying. Details are shown in Table 4.32. The percentage of bulls observed lying differed (P=0.0013) between the years (25.0 vs. 33.2% in year 1 and 2, respectively) but not that of castrates (Table 4.32).

For standing/walking a year x treatment interaction (P<.0001) and an interaction between year and sex (P=0.0023) was found. Only in year 2 more (P<.0001) WbC calves were observed standing/walking than WsC calves (61.5 vs. 52.2%; Table 4.31). In year 1 compared to year 2, less (P=0.0001) WbC calves (53.3 vs. 61.5%) but more (P=0.0439) WsC calves (56.5 vs. 52.2%) were observed standing/walking (Table 4.31). For standing/walking there was no difference (P=0.2076) detected for bulls between the years. However, in year 2 more castrates (P=0.0023) were observed standing/walking than in year 1 (65.9 vs. 59.3%; Table 4.32).

Table 4.32 Trial 3 - Behavior of castrates and bulls (% of calves) (lsmeans $_{s.e.}$) after castration by year

Behavior	Sex	Year 1	Year 2
Lying	Castrates	$16.8^{A}_{1.8}$	$13.1^{A}_{1.8}$
	Bulls	$25.0^{Ba}_{1.8}$	$33.2^{Bb}_{1.8}$
Standing/walking	Castrates	$59.3^{Aa}_{1.5}$	$65.9^{Ab}_{1.5}$
	Bulls	$50.5^{B}_{1.5}$	$47.8^{B}_{1.5}$

A, B = Column values with different capital letters are significantly different (p<0.05) between sexes (Tukey`s test).
a, b = Row values with different small letters are significantly different (p<0.05) between years (Tukey`s test).

Differences between the treatments for standing/walking were detected on d 1 (P=0.0076) and d 3 (P<.0001). On d 1 after castration more WsC than WbC calves (65.0 vs. 57.9%) were standing/walking (Table 4.33). However, on d 3 the opposite was observed, with more (P<.0001) WbC than WsC calves standing/walking (58.7 vs. 45.4%). Further details considering the treatment x day after castration interaction for standing/walking are shown in Table 4.33.

Table 4.33 Trial 3 - Behavior (% of calves) (lsmeans $_{s.e.}$) on d 1, 2 and 3 after castration, when weaned and castrated the same day or weaned 8 weeks before castration

Behavior	Treatment[1)]	Day after castration		
		1	2	3
Feeding	WsC	$17.1^{a}_{1.7}$	$21.7^{a}_{1.7}$	$28.9^{Ab}_{1.7}$
	WbC	$19.8_{1.7}$	$22.1_{1.7}$	$22.9^{B}_{1.7}$
Lying	WsC	$17.9^{a}_{2.2}$	$25.6^{b}_{2.2}$	$25.7^{Ab}_{2.2}$
	WbC	$22.3_{2.2}$	$22.3_{2.2}$	$18.3^{B}_{2.2}$
Standing/walking	WsC	$65.0^{Aa}_{1.9}$	$52.7^{b}_{1.9}$	$45.4^{Ac}_{1.9}$
	WbC	$57.9^{B}_{1.9}$	$55.6_{1.9}$	$58.7^{B}_{1.9}$

A, B = Column values with different capital letters are significantly different (p<0.05) between treatments (Tukey`s test).
a, b, c = Row values with different small letters are significantly different (p<0.05) between days (Tukey`s test).
[1)] WsC = Weaning and castration occurred the same day. WbC = Weaning occurred 8 weeks before castration.

Furthermore, a treatment x day after castration interaction (P=0.0221) for lying was found. Percentage of WbC calves lying was not different between the days after castration (Table 4.33). However, less (P=0.0123) WsC calves were lying on d 1 (17.9%) than on d 2 (25.6%) and less (P=0.0112) WsC calves on d 1 compared to d 3 (25.7%; Table 4.33). On d 3 more (P=0.0164) WsC than WbC calves were lying (25.7 vs. 18.3%), but there were no differences between the treatments on d 1 and d 2.

4.3.4 Trial 3 - Growth Performance after Castration

BW of the calves at T0 (castration and weaning of WsC treatment) and BW at T3 (3 weeks after T0) was not affected by year, treatment or sex. At the beginning of the pasture period (TP) WbC calves were almost 20 kg heavier (P=0.0424) than WsC calves (369.8 vs. 350.9 kg;

Table 4.34). At the same time the WsC calves were about 24 days younger (P<.0001) than the WbC calves (320.6 vs. 344.5 days; Table 4.34).

Table 4.34 Trial 3 - Age (d), body weight (kg), live weight gain (kg/d), and average daily gain (kg/d) (lsmeans $_{s.e.}$) of the calves at different times by effects

Item	Year		Treatment[2)]		Sex	
Time	1	2	WsC	WbC	Castrates	Bulls
Age, d						
at weaning	184.0 $_{3.0}$	184.9 $_{3.6}$	208.6^{a} $_{3.6}$	160.3^{b} $_{3.2}$	184.3 $_{3.3}$	184.5 $_{3.3}$
T0	213.0 $_{3.0}$	212.9 $_{3.6}$	208.4 $_{3.6}$	217.4 $_{3.2}$	212.8 $_{3.3}$	213.0 $_{3.3}$
TP[3)]	337.9^{a} $_{3.0}$	327.3^{b} $_{3.6}$	320.6^{a} $_{3.4}$	344.5^{b} $_{3.2}$	332.5 $_{3.3}$	332.6 $_{3.3}$
Body weight, kg						
T0	256.8 $_{4.8}$	249.9 $_{5.8}$	247.4 $_{5.4}$	259.2 $_{5.1}$	253.5 $_{5.3}$	253.1 $_{5.2}$
T3[4)]	279.4 $_{4.7}$	277.7 $_{5.7}$	275.3 $_{5.4}$	281.9 $_{5.1}$	276.0 $_{5.3}$	281.2 $_{5.2}$
TP	365.1 $_{5.9}$	355.6 $_{7.3}$	350.9^{a} $_{6.7}$	369.8^{b} $_{6.5}$	329.4^{a} $_{6.6}$	391.2^{b} $_{6.7}$
Live weight gain, kg/d						
birth to T0	1.06 $_{0.02}$	1.02 $_{0.02}$	1.02 $_{0.02}$	1.05 $_{0.02}$	1.04 $_{0.02}$	1.04 $_{0.02}$
Average daily gain, kg/d						
T0 to T3	0.90^{a} $_{0.04}$	1.26^{b} $_{0.05}$	1.29^{a} $_{0.05}$	0.87^{b} $_{0.05}$	0.93^{a} $_{0.05}$	1.23^{b} $_{0.05}$
T3 to TP	0.80 $_{0.05}$	0.86 $_{0.07}$	0.86 $_{0.06}$	0.81 $_{0.06}$	0.56^{a} $_{0.06}$	1.10^{b} $_{0.06}$

a, b = Row values with different small letters are significantly different (p<0.05) within effect.
[1)] T0 = Castration date and weaning of WsC treatment.
[2)] WsC = Weaning and castration occurred the same day. WbC = Weaning occurred 8 weeks before castration.
[3)] TP = Beginning of pasture period.
[4)] T3 = T0 + 3 weeks.

At the beginning of TP castrates were more than 30 kg lighter (P<.0001) compared to same-age bulls (Table 4.34). For BW at TP a year x sex interaction was detected with greater (P=0.0161) BW for the bulls in year 1 than year 2. BW of the castrates did not differ between the years at the beginning of TP (Table 4.35).

Table 4.35 Trial 3 - Body weight (kg) (lsmeans $_{s.e.}$) of castrates and bulls at the beginning of pasture period (TP) by year

Item	Sex	Year 1	Year 2
Body weight, TP, kg	Castrates	322.5^{A} $_{8.3}$	336.4^{A} $_{10.4}$
	Bulls	407.6^{Ba} $_{8.5}$	374.8^{Bb} $_{10.4}$

A, B = Column values with different capital letters are significantly different (p<0.05) between sexes (Tukey`s test).
a, b = Row values with different small letters are significantly different (p<0.05) between years (Tukey`s test).

LWG, calculated from birth to T0, was not influenced by any of the effects. However, a year x treatment interaction and an interaction between treatment and sex was found. The WsC calves had a greater (P=0.0008) LWG in year 1 compared to year 2 (1.09 vs. 0.96 kg/d), but no difference for WbC calves between the years was detected (Table 4.36). LWG did not differ between the treatments in year 1. However, in year 2 for the WsC calves less (P<.0001) LWG was found than for WbC calves (0.96 vs. 1.08 kg/d; Table 4.36).

Table 4.36 Trial 3 - Live weight gain (kg/d) and average daily gain (kg/d) (lsmeans $_{s.e.}$) of calves, when weaned and castrated the same day or weaned 8 weeks before castration by year

Item	Treatment[1)]	Year 1	Year 2
LWG[2)], birth to T0, kg/d	WsC	1.09^{a} $_{0.02}$	0.96^{Ab} $_{0.03}$
	WbC	1.03 $_{0.02}$	1.08^{B} $_{0.03}$
ADG[3)], T0 to T3, kg/d	WsC	0.93^{a} $_{0.06}$	1.64^{Ab} $_{0.08}$
	WbC	0.87 $_{0.06}$	0.88^{B} $_{0.07}$

A, B = Column values with different capital letters are significantly different (p<0.05) between treatments (Tukey`s test).
a, b = Row values with different small letters are significantly different (p<0.05) between years (Tukey`s test).
[1)] WsC = Weaning and castration occurred the same day. WbC = Weaning occurred 8 weeks before castration.
[2)] LWG = live weight gain.
[3)] ADG = average daily gain.

For the bulls LWG was different (P=0.0242) between the treatments, with a greater LWG for the WbC bulls compared to the WsC bulls (1.08 vs. 0.99 kg/d). For the castrates, no difference between the treatments was found (Table 4.37).

Table 4.37 Trial 3 - Live weight gain (kg/d) (lsmeans $_{s.e.}$) of castrates and bulls, when weaned and castrated the same day or weaned 8 weeks before castration

Item	Sex	Treatment[1)]	
		WsC	WbC
LWG[2)], birth to T0, kg/d	Castrates	1.05 $_{0.03}$	1.03 $_{0.03}$
	Bulls	0.99^{a} $_{0.03}$	1.08^{b} $_{0.03}$

A, B = Column values with different capital letters are significantly different (p<0.05) between sexes (Tukey`s test).
a, b = Row values with different letters are significantly different (p<0.05) between treatments (Tukey`s test).
[1)] WsC = Weaning and castration occurred the same day. WbC = Weaning occurred 8 weeks before castration.
[2)] LWG = live weight gain.

ADG during 3 weeks following T0 (T0 to T3) was greater (P<.0001) in year 2 than year 1 (1.26 vs. 0.90 kg/d), greater (P<.0001) for WsC compared to WbC calves (1.29 vs. 0.87 kg/d), and smaller (P<.0001) for the castrates than for the bulls (0.93 vs. 1.23 kg/d; Table 4.34). Furthermore, there was a year x treatment interaction found for this growth rate. ADG from T0 to T3 of WsC calves was smaller (P<.0001) in year 1 than in year 2 (0.93 vs. 1.64 kg/d; Table 4.36). There was no difference found for WbC calves between the years. Within year 2, the WsC calves had greater (P<.0001) ADG than the WbC calves (1.64 vs. 0.88 kg/d) but no treatment difference was detected in year 1 (Table 4.36). ADG during the pre-pasture period (T3 to TP) was about twice as high (P<.0001) for the bulls than for the castrates (1.10 vs. 0.56 kg/d). However, ADG during that period was not affected by year or treatment (Table 4.34).

4.3.5 Trial 3 - Slaughter Traits

Slaughter traits were recorded for year 1 only. There was no treatment effect on any of the carcass traits (Table 4.38). At slaughter, the castrates were about 7.5 months older (P<.0001) and more than 120 kg heavier (P<.0001) than the bulls (Table 4.38). However, LWG, calculated from birth to slaughter, was greater (P=0.0034) for the bulls than for the castrates (0.93 vs. 0.78 kg/d; Table 4.38).

Table 4.38 Trial 3 - Age (d), body weight (kg) and live weight gain (kg/d) (lsmeans $_{s.e.}$) at slaughter of castrates and bulls, when weaned and castrated the same day or weaned 8 weeks before castration

Item	Treatment[1)]		Sex	
	WsC	WbC	Castrates	Bulls
Slaughter age, d	547.0 $_{17.1}$	546.7 $_{14.4}$	659.4^{a} $_{16.4}$	434.3^{b} $_{14.6}$
Final body weight, kg	474.1 $_{12.4}$	475.5 $_{8.0}$	536.4^{a} $_{9.4}$	413.3^{b} $_{10.6}$
Live weight gain, kg/d				
birth to slaughter	0.87 $_{0.04}$	0.85 $_{0.03}$	0.78^{a} $_{0.03}$	0.93^{b} $_{0.4}$

a, b = Row values with different small letters are significantly different (p<0.05) between treatments / sexes (Tukey`s test).
[1)] WsC = Weaning and castration occurred the same day. WbC = Weaning occurred 8 weeks before castration.

5 GENERAL DISCUSSION

The Burdizzo castration was performed in the context of this thesis because it is the most common method to castrate cattle in Germany. For the same reason abrupt separation was conducted in the present trials. It is the most frequently exercised weaning strategy in cow-calf-production systems.

5.1 Vocalization

When suddenly separated from their mothers, beef calves at an age of 2 to 6 months produce a lot of vocalizations and show other signs of distress (Spinka, 2006). After abrupt separation cows and calves vocalized repeatedly and spent more time walking, while spending less time feeding and resting (Veissier and Le Neindre, 1989).

5.1.1 Time and Age

There was an effect of time on vocalization activity of the calves found in all trials of this thesis. The number of vocalizations decreased in the course of the days after weaning in Trial 1 and 2. In Trial 3, there was a reduction of vocalization from d 1 to d 3 after castration + weaning (treatment WsC) as well as after castration (analysis treatments WsC and WbC year 2). Vocalization activity of the offspring following weaning is characterized by a decrease in the course of the days following weaning; not only observed in cattle, but also in sheep (Schichowski et al., 2010) and pigs (Weary and Fraser, 1997). As vocalization can be a useful indicator of welfare (Watts and Stookey, 2000), the calves seemed to get more and more accustomed to their new social and physical environment after separation from their dams during the course of the days of the trials.

In Trial 1, the younger W6 calves, weaned at 6 months of age, vocalized more than the calves of the older weaning age (W8). This is in agreement with the findings of Smith et al. (2003), who observed calves weaned at 4 and 7 months of age, respectively. The impact of weaning age on the vocalization activity of the young was also found in other species than cattle (Schichowski et al., 2010; Weary and Fraser, 1997). Similarly, the younger age of the calves in all probability was the reason, that the WsC calves vocalized more than the WaC calves after weaning (Trial 2). The weaning ages of the W8 (8.0 months) and WaC calves (7.4 months) were closer to the natural weaning age of about 10 months (Reinhardt and Reinhardt, 1981), compared to the weaning age of about 6 months, that occurred for the treatments W6

(Trial 1) and WsC (Trial 2). The older calves were not as much impacted by the weaning procedure, including the withdrawal of milk supply and separation from the dam. Flower and Weary (2003) reported, that calves will typically continue to receive some milk from the dam until 6 to 9 months of age, when cow and calf are kept under naturalistic conditions (cited in Weary et al., 2008). In Trial 1, the younger calves (W6) vocalized more than the older calves (W8) on each of the three days after weaning. For both weaning ages there was a decrease in the course of the days, but for the W8 calves vocalization was more reduced from d 1 to d 2 (45.4% of calls on d 1) and to d 3 (13.9% of calls on d 1). W6 calves seemed to suffer more and longer from weaning stress, because for them there was a smaller decrease of the number of calls from d 1 to d 2 (57.3% of calls on d 1) and to d 3 (18.7% of calls on d 1). This result is in accordance to Smith et al. (2003). Similar findings were shown by an interaction between treatment and day after weaning (Trial 2). Thereby, on d 2 after weaning, the number of vocalizations of the younger WsC calves was reduced to 38.1% in relation to d 1, while on d 3 these calves vocalized 11.2% of the calls in relation to d 1. This was a smaller reduction of the vocalization counts compared to the older WaC calves (from 100% on d 1 to 24.7% on d 2 and only 5.8% on d 3). These results indicated, that the WaC calves became acquainted with the new environment after weaning more quickly. They did not suffer from weaning distress with the same intensity as the WsC calves. The treatment x sex interaction detected in Trial 2 also indicated an effect of age on the weaning-induced vocalization. Within both sexes, independent from castration, the younger WsC (weaned at 6.5 months of age) vocalized more than the older WaC calves (weaned at 7.4 months of age).

5.1.2 Female - Male

In Trial 1, female calves showed a higher vocalization activity than male calves, possibly indicating that female calves suffered more from weaning stress. Based on the different natural weaning ages of female (8.8 months of age) and male calves (11.3 months of age) found by Reinhardt and Reinhardt (1981), this finding contrasted to expectations. Because of the older natural weaning age of male calves, one could have expected a stronger impact of weaning distress on male than female calves in this trial, that resulted in a higher vocalization activity in the male calves. On the other hand, in a study about white tailed deer (*Odocoileus virginianus*), Schwede et al. (1994) observed that males are associated less and more loosely with their mothers than females. From a study including 11 Salers cow-calf pairs, Veissier et al. (1990b) reported that the female yearlings were closer to their dams compared to the male young. In Trial 1 of the present thesis, female vocalized more than male calves on d 1 after weaning. However, the number of vocalizations of female calves decreased more from d 1 to d 2 (50.6% of d 1), compared to the vocalization activity of the male calves that had on d 2, 55.2% of the calls of d 1. This sex difference in terms of the intensity of vocalization in Trial 1 suggested that the male calves probably suffer more from the separation from their dams. These findings agreed with Hickey et al. (2003), who analyzed high values of noradrenalin and fibrinogen in the blood of the bull calves on d 7 after weaning, indicating that the males are more influenced by the weaning distress than the females. In Trial 1, the higher vocalization activity of the female calves mentioned before was possibly more a reflection of the females` higher propensity to communicate by vocalization rather than a single stress response. Watts et al. (2001b) observed more female than male weaned calves vocalizing during 1-min visual isolation, tested when the calves were from 8-12 and 11-15 months old, respectively.

5.1.3 Castration

The findings from the analysis of vocalization after castration (Trial 3) indicated that calves' calls were more motivated by weaning distress and less a stress response initiated by castration. The WbC calves (0.01 calls/10min) that were weaned 8 weeks before castration and observation showed very low vocalization activity, whereas the WsC calves, weaned at castration, vocalized more than a hundred times as often (1.12 calls/10 min). Equivalent, the vocalization activity after castration + weaning (treatment WsC Trial 3) was greater for bulls than castrates. This confirmed that vocalization was more motivated by weaning distress than castration pain. In Trial 3, additional impact of castration on newly weaned calves (treatment WsC) did not increase the number of vocalizations. On the contrary, vocalization activity was lowered by castration. It is known, that there is a swelling of the local tissue around castration site following Burdizzo castration (Molony et al., 1995). This is a physiological reaction on the crushing with the Burdizzo clamps to destroy the spermatic cords carrying blood to the testicles. Vocalizing a call is physically going along with an exertion of the abdomen muscles of the animal. Such an exertion of the abdomen muscles has an impact on the local tissue of the castration site, causing discomfort or pain. Because of such physiological suffering, the newly castrated and weaned calves possibly avoided calling and showed a smaller vocalization activity than the recently weaned intact bulls, which were exposed only to weaning distress, but not to physical disturbance of the body. Such prevention behavior displayed by the castrates was also indicated by castrates vocalizing less than bulls on d 2 (treatment WsC Trial 3). This difference between the sexes was caused by a stronger decrease of the castrates` vocalizations from d 1 to d 2 after castration + weaning. On d 2, WsC castrates vocalized only 37.2% of the number of calls of d 1, whereas WsC bulls on d 2 still vocalized 66.5% of the calls of d 1.

In Trial 2, the bulls showed a higher number of vocalizations after weaning compared to the castrates. The assumption concerning the reduction of the vocalization activity by castration was confirmed by an interaction between treatment and sex detected in Trial 2. There was the same level of vocalization activity after weaning found for castrates and bulls of the WaC treatment. Weaning of these WaC calves occurred 4 weeks after castration. The physical impact of castration (swelling and inflammation) was minimized meanwhile and therefore did not influence vocalization behavior of the WaC castrates at this time.

Because the sex of the calves did not influence vocalization after castration in Trial 3, it can be assumed that vocalization activity was an improper indicator for castration stress or suffering in the context of this trial. However, the intensity of weaning stress may be derived from the vocalization activity of the newly weaned calves, particularly because there was a decrease of vocalization counts in the course of the days after weaning. As discussed before, such reduction of vocalization activity after weaning was also observed by other studies.

5.1.4 Individuality and Group Size

The very small difference (P=0.0489) between the replications in Trial 1 probably was caused by the individuality of the calves. The propensity of cattle to vocalize may be a persistent individual characteristic (Watts and Stookey, 2001). From observations of cow-calf pairs in extensive range conditions, Watts et al. (2001b) found that the number of vocalizations given by individual cows and their own calves were highly correlated. In their study on 17 full-sibling families of beef calves, Watts et al. (2001a) found a significant influence of sire and family on the number of vocalizations. In Trial 2, the difference between year 1 and year 2 was also potentially caused by the individuality of the calves. In this context one should keep in mind that 25% of the animals observed in year 2 were pure Aubrac cattle, whereas all calves in year 1 were crossbreds. Watts (2001) presumed, that the observed vocal characteristic was partly genetically inherited because vocalization varied due to sire and family. In an isolation test with adult cattle, Le Neindre (1989) found Salers vocalizing more than Friesians.

On each of the three days after castration + weaning calves vocalized more in year 1 than in year 2 (Trial 3, treatment WsC). The less vocalization activity in year 2 could be caused by the lower number of calves in the groups examined in year 2. In smaller groups, calves do not need such a long time to establish a new social structure in comparison to a bigger group size. Group size plays a role in the time required to establish the social hierarchy of the group (Ingrand, 2000). Therefore, the calves in the smaller group were potentially not distressed to the same extent by the separation from their dams and former herd mates and the resulting necessitated social restructuring, compared to the newly weaned calves managed in lager groups.

It can be concluded, that under the conditions of the present thesis, age and sex of the calf, the physical discomfort by recently performed castration, the individuality of the animal and also the group size had a potential impact on the vocalization activity of the calves.

5.2 Behavior

5.2.1 Behavior Type Feeding

In the trials of the present thesis, feeding behavior was defined as percentage of calves observed standing within feeding area. In Trial 1, feeding behavior observed after weaning was not influenced by any of the effects or interactions between them. Unlike this, in Trial 2, differences between the days after weaning in terms of feeding behavior appeared. Thereby, the lowest percentage of calves feeding was observed on d 2 (13.1%) and the highest on d 3 (23.9%), with d 1 (17.3%) intermediate. Concerning the time spent feeding, Price et al. (2003) also observed the lowest value on d 2 (20 to 30 h after weaning) compared to d 3 and d 4, while d 1 and d 5 ranged in the middle and both did not differ from any other day.

In Trial 2, no effect of treatment or sex on feeding behavior after weaning was detected. These results indicated, that the newly weaned calves did not suffer from additional stress caused by castration in terms of feeding behavior. In a study performing surgical castration, Daniels et al. (2000) also did not find an impact of timing of castration in relation to the arrival in the feedlot, on feeding and watering behavior.

In Trial 3, feeding behavior after castration was observed with an increase of the percentage of calves feeding from d 1 to d 3. However, castration had no impact on feeding behavior, as there was no significant difference between castrates and bulls. In contrast, Fisher et al. (1996) found that 5.5 months old Burdizzo castrated Friesian bull calves had reduced feed intake compared to intact bull calves in the period from 1 to 5 days after treatment.

In a study evaluating different weaning techniques, Price et al. (2003) found a decrease in time spent eating as an indicator for weaning distress. Based on this, it can be presumed that the more frequently observed feeding behavior in the course of the days following castration in Trial 3 reflected an increasing well-being of the calves.

Although there was no treatment effect on feeding behavior in Trial 3, WbC and WsC calves showed different behavior patterns in the course of the observation period. Thereby, WsC calves were increasingly engaged in feeding, whereas the percentage of WbC calves observed feeding did not differ between the days. The increase in the percentage of WsC calves observed feeding was potentially a reflection of a compensation behavior of these calves. The WsC calves were newly weaned at castration. They might have been exposed to a deficit in energy supply before weaning due to milk deprivation and / or an insufficient mobilization of nutrients from solid feed. Therefore, after such a period of low energy input, these calves potentially compensated by increasing feeding behavior in the course of the days following weaning. This observed WsC behavior is in agreement with Gibb et al. (2000), who reported an increasing number of newly weaned beef calves observed eating from an average of 17.4% on the first 2 days to an average of 27.6% on d 4, 5 and 6 after arrival in the feedlot. In contrast, the WbC calves were already adapted to the weaning status and on solid feed at the time of observation after castration. Therefore, WbC calves` feeding behavior did not change in the course of the days after castration. In this trial, the compensation behavior of the newly weaned WsC calves, resulted to significant more WsC calves observed feeding than previously weaned WbC calves, on d 3 after castration.

Another influencing factor in the context of feeding behavior is the social environment. In Trial 3, the social environment was new for the WsC calves, while the social hierarchy of the WbC calves had already been established. Weaning implies for the calves to leave a group with adults and to be mixed with animals of their own age. Such removal of the young out of a mixed-age group can have an impact on the feeding behavior of the young. Krohn and Konggaard (1979) found young first lactation cows spending 10 – 15% more time eating when kept separated from older cows compared to managing them in a mixed group with older multiparous cows.

In Trial 3, more calves showed feeding behavior in year 1 than year 2. This was potentially result of a larger group size in year 1. Less feeding space per calf supposedly led to a higher replacement of calves visiting the feeders due to calves pushing each other aside. Such replacements potentially resulted in more feeding bouts per animal with a shorter duration per bout at the feeders. As a consequence of this, more calves were observed in the feeding area. In contrast to this consideration, DeVries and von Keyserlingk (2009) reported about competitively fed heifers (2 heifers/feed bin). They tended to have 10% shorter feeding times and consumed 9% fewer meals per day, although meals were of longer duration (10%) and tended to be larger (13%), compared to the heifers of the non-competitive group (1 heifer/feed bin). The authors concluded that the growing heifers` competition for feed tended to increase the day-to-day variation in feeding behavior. Beside such replacements at the feeders, in year 1, more calves could have been observed feeding, defined as standing within the feeding area, because there was in general less space per animal in that year. Ingrand (2000) reported that group size have little impact on feeding behavior, but it influences the time required to establish social hierarchy of the group. Based on this fact, it might be possible, that the feeding behavior observed in Trial 3, was confounded with social encounters between the calves as part of the group hierarchy formation.

5.2.2 Behavior Types Lying and Standing/Walking

In Trial 1, female and male calves did not differ in terms of lying and standing/walking, respectively. Although, concerning the higher vocalization activity of the females, it could have been expected that the female calves showed restlessness resulting from weaning distress to a higher extent than the male calves.

The younger calves obviously were more restless compared to the older calves, as more W8 than W6 calves were observed lying. In contrast, less W8 than W6 calves were observed standing/walking. Due to the fact that more W6 than W8 calves showed non-resting behavior, it can be assumed that weaning affected the younger calves more. Smith et al. (2003) also observed more locomotion in early weaned (4 months) than later weaned (7 months) calves.
Another indicator for the older calves recovering faster from weaning distress was displayed by the interaction between weaning age and day after weaning, detected for lying behavior in Trial 1. Thereby, there was no difference between W6 and W8 calves on d 1 and d 3, but more W8 than W6 calves were lying on d 2. This was caused by a very high increase of W8 calves observed lying from d 1 to d 2, whereas lower increase was found for the younger W6 calves.

In Trial 1, the percentage of calves observed lying increased in the course of the days following weaning. The opposite was found in terms of standing/walking. These findings potentially reflected the increasing adaption of the newly weaned calves to their new environment. Veissier et al. (1989) reported similar findings.

Slightly different to the observations in the course of the days in Trial 1 was the calves` lying behavior after weaning in Trial 2. There was a high increase in the percentage of calves lying from d 1 to d 2 after weaning, but no difference between d 2 and d 3. In contrast, the decrease of the percentage of calves observed standing/walking was more continuous. The different behavior pattern in Trial 2 for lying in the course of the days could indicate that the additional stressor castration, that occurred only in Trial 2 but not in Trial 1, influenced the resting behavior of the calves following weaning.

More WsC than WaC calves were lying after weaning, even though WsC calves vocalized more than WaC calves. Therefore, the resting behavior of the newly weaned WsC calves in

Trial 2, was potentially more an indication of exhaustion rather than well-being resulting from low-stress conditions for the calves. The exhaustion of the WsC calves was most likely caused by vocalization and weaning distress and, in terms of the WsC castrates, also by a physical suffering from the castration. For the WsC castrates, the castration procedure was performed on the same day as weaning. In contrast, when observed following weaning, the WaC calves suffered only from weaning distress but no longer from physical pain, as the castration procedure was applied 4 weeks before weaning. Therefore, the WaC castrates most likely were in better physical condition at weaning because they had time to recover from castration. A treatment x day after weaning interaction was detected in Trial 2 that potentially confirmed the assumption, WsC calves` lying behavior would have been result of exhaustion. On d 1, more than twice as many WsC calves were lying compared to WaC calves. Whereas on the following observation day no significant differences between WsC and WaC appeared due to increased lying of WaC calves.

In Trial 2, no difference between castrates and intact bulls was detected for lying behavior after weaning. For the WaC calves this finding allows the conclusion that the Burdizzo castration had no long-term influence on the lying behavior of the calves. In terms of the WsC calves, the absence of a sex effect indicated that the possible impact of castration on the lying behavior was super-imposed by the influence of weaning under the conditions of this trial. Furthermore, the absence of a difference between the sexes in terms of lying behavior confirmed the assumption that exhaustion had an impact on WsC calves` lying.

For the behavior type standing/walking, an interaction between treatment and day after weaning was detected in Trial 2. More WaC than WsC calves were observed standing/walking on d 1, while only a slight decrease was noticed in the WsC calves from d 1 to d 2, with d 3 at the same level as d 2. This behavior of the WsC calves decreased not in the same manner as the more continuous decrease from d 1 to d 3 after weaning, that was observed for the WaC calves and also for the calves in Trial 1. Therefore it can be assumed that the additional stressor castration, imposed on the WsC castrates in Trial 2, caused this changed behavior pattern. However, because of the absence of a treatment x sex interaction, it has to be concluded, that castration and weaning were confounded with regard to their impact on the calves` behavior under the conditions of this trial.

In Trial 3, when behavior after castration was observed, almost twice as many bulls than castrates were lying, while on the other hand more castrates than intact bulls were observed standing/walking. These findings potentially indicated a negative impact of castration on

resting behavior. Similar, Molony et al. (1995) observed in castrates, compared to intact male control calves, an increasing time spent abnormal standing following castration. In all probability, it was painful or at least discomforting for the recently castrated calves to lay down on the concrete surface. Consequently they avoided lying behavior.
In Trial 2, nearly half of all WsC were observed lying following weaning (and castration), whereas in Trial 3, only just under 25% of the WsC calves were lying. This trial difference is most likely due to the different housing conditions, such as the straw bedding in Trial 2.

Treatment as well as day after castration did not affect lying behavior, indicating that the impact of weaning distress (WsC calves) on this type of behavior was not detectable under the conditions of Trial 3. However, a treatment x day after castration interaction was detected, showing, that lying behavior of the WbC calves did not change in the course of the days, whereas the percentage of WsC calves observed lying increased from d 1 to d 2 and remained at this level on d 3. The previously (8 weeks before) weaned WbC calves were most likely more settled because they lived in an established social environment and did not suffer from weaning distress anymore, as indicated by the absence of vocalization activity. On the other hand, the increased lying behavior observed for WsC calves is not unusual for newly weaned calves. Haigh et al. (1996) reported about increased lying being used as an indicator for reduced stress in weaned animals (cited in Gibb et al., 2000).
Interestingly, more WsC than WbC calves were lying on d 3, potentially because they were exhausted from their vocalization activity and increased feeding behavior for compensation reasons and therefore had an increased need to rest. The possible impact of exhaustion on the behavior of the WsC calves was also discussed before, in relation to Trial 2.

In Trial 3, significantly more WbC than WsC calves were observed standing/walking after castration, potentially indicating that the additional impact of weaning at castration influenced the activity of the WsC calves by weakening the effect of castration. However, at this point, the effects of weaning and castration on the behavior cannot clearly be differentiated because of the absence of an interaction between sex and treatment. Similar, as discussed before with regard to lying behavior, one could presume that less standing/walking of the WsC calves after castration is rather an expression of exhaustion than of well-being. Further indications for this assumption were shown by a treatment x day after castration interaction for standing/walking, that had similar patterns, but converse values compared to the lying behavior in Trial 3.

In Trial 3, more bulls were observed lying in year 2 compared to year 1, probably because of the smaller group size in year 2 resulting in more floor space available for each calf. However, even though more space was usable for each individual, not more castrates were observed lying in year 2 than year 1. This indicated that, regardless of the floor space allowance, the castrates avoided lying on the floor after castration under the conditions of this trial. On the other hand, the greater floor space offered in year 2 led to more castrates standing/walking in year 2 compared to year 1. This higher standing/walking behavior shown by the castrates was potentially due to castration, as the standing/walking behavior of the intact bulls was not significantly different between the years and floor space per animal, respectively. Independent from the floor space available for each calf, a higher percentage of castrates than intact bulls was observed standing/walking in both years. Molony et al. (1995) observed a similar increase in time spent abnormal standing and total statue standing in Burdizzo castrated calves compared with the non-castrated control calves.

5.3 Blood Traits

Blood traits were evaluated only in Trial 2 of this thesis. Blood sampling at T0 (castration and weaning WsC calves) was conducted to get information from the blood about the health status of the calves before any of the trial-induced stressors, castration and weaning, occurred. Merck Veterinary Manual (2006) was used as reference in terms of blood traits, exclusive of Haptoglobin.

5.3.1 Haptoglobin

Castration did not affect Haptoglobin concentration under the conditions of this trial. Although at T1, which was one week following T0, an increased value was found in the castrates compared to the bulls, no significant difference between the sexes was detected.

A significant difference between WsC and WaC calves, surprisingly, only appeared at T0. The WaC calves had a greater Haptoglobin concentration than the WsC calves, potentially caused by stress and enragement (shown by the other animals) due to weaning and castration procedures at T0. All handling procedures (including getting out of the stable and short-time separation from the dams) as well as the environment (corral, weighing facility and head gate) and the keepers were new for the calves. In both years, at T0, on the firstly handled calves castration and also weaning occurred (WsC castrates), while in one of the years, the secondly handled animal group consisted of WsC bulls. Therefore, the WaC calves had time to recognize and possibly catch the stress that affected these previously handled calves and the corresponding dams as well. Haptoglobin potentially indicated this (psychological) stress of the WaC calves, because this major acute-phase protein increases within a few hours following an imbalance of the homeostasis (Carroll et al., 2009).

By unknown reasons, at T0, Haptoglobin concentration was more than twice as high in year 2 than in year 1. Concerning these findings, it could be assumed that, under the conditions of this trial, the acute-phase protein Haptoglobin concentration was not suitable to display the impact of the two stressors (weaning and castration), alone or in combination, on the calves. On the other hand, it should be kept in mind that Haptoglobin is the product of the very sensitive, but non-specific acute-phase response. Therefore, Haptoglobin in this trial possibly was too sensitive to differentially indicate if there was only a single stressor or two combined stressors affecting the health status of the calves. Similarly, Hickey et al. (2003) did not find an effect of abrupt weaning, sex (male, female) or sampling time on Haptoglobin. But in contrast to the present trial, these calves were habituated to handling.

Because the Haptoglobin production can be influenced by a number of factors, the differences detected between the years at T4 and T5 cannot be explained conclusively. An infection of the respiratory tract, that was treated in two calves from the WaC treatment in year 1 following T4, could be one possible reason. However, at T4 and T5, no treatment differences were detected, that would have been induced by such infection and therefore could affirm that hypothesis.

5.3.2 White Blood Cell Counts

The absence of a treatment effect on the total WBC number indicated that castration and weaning, occurring separately as well as in combination, did not affect the response of the calves in terms of this blood trait, under the condition of this trial. Similarly, in a study to examine the effect of abrupt separation on male and female calves, Hickey et al. (2003) did not find a weaning effect on total white blood cell concentrations. However, the authors reported an increase of the total leukocyte concentration, found at 24 h and 48 h, but not at 168 h following the social group disruption. Therefore, it could be presumed, that a different blood sampling time scheme, closer to the stressful event, as applied in the present thesis, would have been more suitable to detect a possible WBC change due to weaning.

However, castration potentially increased the WBC concentration, as there was as greater WBC number in castrates than bulls at T2 and T3, which were 14 and 21 days following castration, respectively. Interestingly, the WBC counts of castrated and non-castrated calves were not different at T4. However, subsequently, at T5 (49 days after castration), the castrates also had a greater WBC number than the bulls. As Ting et al. (2005) found a significantly greater WBC number in Burdizzo-castrated calves than in intact male calves only on d 2 after castration, it has to be presumed that, besides the castration, further factors increased the WBC number in the present thesis. For example, one possible factor could be the infection, as was mentioned in the discussion of the Haptoglobin concentration. Recently castrated calves are potentially more fragile to get infected than intact bulls.

5.3.3 Neutrophils and Lymphocytes

A neutrophil is a white blood cell that increases in response to bacterial infection and stress. Weaning increased neutrophils` proportion at 24 and 168 h after weaning in male and female calves (Hickey et al., 2003). In the present trial, the number of neutrophils was higher in WaC than WsC calves at T4 and also at T5. However, the increase in neutrophils at T4, could not have been triggered by the physical disruption of the cow-calf bonds, because the blood sampling at T4 occurred before the separation of the WaC calves from their dams. Several reasons for the difference between treatments at T4 can be assumed. The main reason for the increase of the number of neutrophils at this time potentially was the infection of the respiratory tract, that appeared in the WaC calves (paragraphs 5.3.1. and 5.3.2.). However, the increased number of neutrophils in WaC calves at T5, could also be caused by weaning, as in accordance to the findings of Hickey et al. (2003).

Interestingly, there was an interaction between year and treatment at T3, T4 and T5. In year 1, the WsC had a lower neutrophil number than WaC calves on each of these sampling points. Based on Holsteg (2002), who found an increasing number of neutrophils indicating stress, it can be suggested that the WsC calves were only mildly stressed by the simultaneous impact of weaning and castration. On the other hand, a decrease of the number of neutrophils can be caused by viral infections. Following that, the low number of neutrophils in WsC calves discussed before could also have been a result of a (subclinical) viral infection. However, conversely in year 2, the WsC calves had a higher number of neutrophils compared to the WaC calves, whereas only at T3, a significant difference between the treatments appeared. Otherwise, an infection of the respiratory tract affected the WaC calves in year 1 and was treated in two animals following T4. Based on this fact, it can be presumed that this infection caused the increased number of neutrophils in the WaC calves in year 1. Considering this, the higher neutrophil values observed in WsC calves compared to WaC calves in year 2 could indicate that there possibly was a greater challenge on the immune system of the WsC calves. In that case, this challenge might have occurred when weaning and castration was performed on the same day. However, in that year, only at T3 a significant difference between WsC and WaC calves occurred.

In year 1, the percentage of neutrophils clearly reflected the good condition of the WsC calves following T0 compared to the WaC calves. In the blood of WsC calves, a significantly lower percentage of neutrophils was found at T3, T4 and T5 compared to WaC calves. Thereby, the percentage of neutrophils in the WsC calves was reduced successively from T3 to T5, whereas the values of the WaC remained at the same level. However, in year 2, no differences were found between the treatments in terms of the percentage of neutrophils.

The number and percentage of neutrophils were not affected by sex, but, interestingly, there were year x sex interactions detected at T2 and T3. For example, at T2 in year 1, the number of neutrophils was significantly higher for castrates than bulls, whereas in year 2 the castrates had a lower number of neutrophils than the bulls, but this difference was insignificant. However, although there was a noticeable difference between the years for the castrates, there was none for the bulls. Furthermore, at T2 in year 1, the percentage of neutrophils was higher in the castrates than the bulls, but this difference was insignificant. However, in year 2, a significantly lower value was found in castrates compared to intact bulls. Therefore, it can be presumed that castration-induced changes in neutrophils concentration could not clearly be detected under the conditions of this trial, potentially due to the additional impact of weaning distress or an unknown factor. Macaulay et al. (1986) studied the responses of 3-weeks old Friesian calves on different castration methods and found 2 hours following castration 44% neutrophils in Burdizzo and 56% neutrophils in surgically castrated, but only 33% neutrophils in non-castrated male calves.

The number of lymphocytes was found to be reduced due to stress (Holsteg, 2002), while the proportion of lymphocytes was increased by weaning up to 7 days following weaning (Hickey et al., 2003). The percentage of lymphocytes was reduced by transportation (Earley and O`Riordan, 2006) and a decrease of T-Lymphocytes population was found in Burdizzo-castrated calves (Murata and Takahashi, 1998).
In contrast to that, in the present trial, the number of lymphocytes was significantly higher for castrates than for bulls at T2, T3, T4 and T5. Although there was no influence of year or treatment at any of the sampling times, a year x treatment interaction was found for the number of lymphocytes. In year 1 at T5, which was one week following weaning of the WaC calves, these calves had a lower number of lymphocytes compared to the WsC calves, potentially due to the weaning stress. However, in year 2 there was no significant difference between the treatments. Because of these results, one could suppose, that the WaC calves in year 1 were more stressed by weaning than the WaC calves in year 2. On the other hand, the lower percentage of lymphocytes in WaC calves, observed at T5 in year 1, potentially indicated the overcoming of the infection that appeared one week earlier (T4) and had to be treated in two WaC calves.

It can be concluded that the observed blood traits, in combination with the applied blood sampling scheme, could not clearly differentiate the stressors weaning and castration, in particular, if additional immune-system-influencing factors occurred, such as infections.

5.4 Growth Performance

In Trial 1, there was no difference between the weaning ages for short-time growth performance related to ADG during 2 weeks after weaning. This potentially resulted from compensatory growth of the W8 calves during the 2 weeks following weaning. The W8 calves achieved an increasing growth rate from the 1-week period before (0.18 kg/d) to the 2-weeks period after weaning (0.76 kg/d), whereas the growth rate of the younger-weaned W6 calves was regressive (1.29 and 0.61 kg/d during 1-pre-weaning week and 2-post-weaning weeks, respectively). These different developments of weight gains indicated that the weaning distress potentially resulted in reduced growth performance in the younger but not in the older calves, weaned at 8 months of age (W8).

The W8 calves had an advantage over the W6 calves in terms of weight gain during a 7-weeks period (from 6 to 8 months of age) before their own weaning. The W8 calves potentially achieved this higher growth rate, because they still remained with their dams and therefore were able to suckle milk from them, while the W6 calves were weaned at the beginning of this growth period. The W8 calves also had a greater LWG, calculated from birth to 8 months of age, compared to the W6 calves, that resulted in a significant greater 8-months BW of the older weaned calves. Based on these findings, it can be assumed, regarding calves` performance up to 8 months of age, that the late weaning at 8 months of age should be preferred under the conditions of Trial 1.

Although there was no significant effect of replication on any of the performance traits, the W8 calves achieved a greater weaning weight in replication 1 than in replication 2. This could have been caused by better energy supply of the suckled W8 calves till their weaning in replication 1 (at 10th of October) compared to replication 2 (at 1st of November). As the pasture period of W8 calves of replication 2 reached further into autumn, the grass potentially had lower energy content or feeding value at this time than some weeks earlier (Klapp, 1971), while, in general, the grass growth declines at this time of the year (Sahin, 2005; Orr et al., 1988). Both factors had potentially a reducing impact on the cows` milk production. In contrast, the weaning weight of the W6 calves did not differ between the replications. These calves had a shorter suckling period at the pasture, lasting till 23th of August and 13th of September for replication 1 and 2, respectively.

LWG, calculated until 6 months of age was not significantly different between W6 and W8. However, a weaning age x replication interaction showed a significantly greater LWG for the

W8 than the W6 calves in replication 1. By this interaction, a further indication for less energy supply through milk suckling was displayed for W8 calves in replication 2 compared to replication 1. Thereby, the LWG till 6 months of age, calculated for the W8 calves, was lower in replication 2 than in replication 1, whereas the W6 calves` LWG was not significantly different between the replications.

The inadequate food supply through milk suckling for the older calves is possibly also indicated by the short-time performance observed prior to weaning. The W6 calves had a significantly greater ADG during 1 week before weaning than the W8 calves.

Potentially due to the better pasture conditions, induced by the time of the year, a greater LWG, calculated from birth to 8 months, was achieved by the W8 calves compared to the W6 calves in replication 1. However, in replication 2, under possibly poorer pasture conditions with lower content and less amount of grass, this growth rate was not significantly different between the weaning ages.

In Trial 1, there was an effect of sex on each of the body weights recorded before and after weaning, where male calves were heavier than female calves at each weighing. Furthermore, LWG until 6 and 8 months of age, respectively, ADG during 2 weeks after weaning and also ADG between 6 and 8 months of age were greater for the males than females. Such sex differences are not unusual due to the different growth potential of female and male calves. This was found by a number of studies, such as Gregory et al. (1991) and Bogart et al. (1963).

Interestingly, an interaction between replication and sex was detected for ADG, calculated from 6 to 8 months, that showed a greater weight gain of the male calves compared to the female calves in replication 1, whereas in replication 2 were no difference between the sexes. Furthermore, male calves had greater weight gains during this period in replication 1 compared to replication 2, while female calves contrasted having greater ADG in replication 2 than in replication 1. Potential reasons for this could have been individual feed intake, feed conversion and growth potential of the calves in combination with milk yield of the dams or weaning distress.

In Trial 2, no influence of any of the effects was found on the BW of the calves at any time before and after castration (T0). However, because of unknown reasons, the WsC calves had a greater weight loss than the WaC calves during the last week before T0 (weaning of WsC and time of castration). One week before T0, all calves together with their dams were brought off the pasture and housed in different pens in the cows` stable. Therefore, the physical environment of the calves changed radically. They were kept indoors for the first time of their life. Furthermore, the social environment of all calves changed also, as due to the arrangement of animals according to the treatment groups, bonds between friendly and related herd mates may have been broken. Regrouping of the cattle most likely resulted in stress also for the adult cattle. Within the new groups, the animals had to establish a social hierarchy. All these factors were potential stressors impacting on the growth performance of the calves. Differences between treatments might be due to the individuality of the animals, possibly particularly in terms of stress tolerance.

ADG during 3 weeks following T0 was greater for WsC than WaC calves, potentially indicating that the supply of nutrients and energy was more adequate for the weaned WsC calves than for the suckled WaC calves. Interestingly, the growth performance was conversely during the 3-weeks period following weaning of treatment WaC (at T4), with WaC calves having a greater ADG than WsC calves.

The very low ADG of the WsC calves during the 3-weeks period following T4 (four weeks after T0) indicated, that the stressors castration and weaning could have had a negative long-term effect on the WsC calves with regard to growth performance. Fisher et al. (1996) found during the third week following castration a significant lower ADG for castrates than for bulls. On the other hand, the WaC calves, being suckled and remaining with their dams for 4 weeks following castration, had an advantage over the WsC calves in terms of ADG during 3 weeks following their weaning at T4. Consequently, at 7 weeks after T0, the BW of the WaC calves was greater compared to that of the WsC calves. However, this treatment difference was not significant.

ADG during 3 weeks following T0 was also greater in year 2 than in year 1. The higher ADG of this period found in year 2, could have been caused by the high growth potential of the purebred Aubrac calves observed in year 2, as 8 of 32 calves examined in that year were Aubracs. BDF (1996) described the characteristics of the cattle breed Aubrac, that have been selected for a number of years under extensive conditions, male calves having 252 kg body weight at an age of 200 d. Based on this, male Aubracs have the growth potential to achieve an ADG of nearly 1.1 kg/d at 200 days of age. In accordance to the information given by the Irish Aubrac Cattle Breed Society (2010), bull calves` body weight at an age of 210 days is 300 kg that equates to an ADG of about 1.25 kg/d. However, irrespective the breed, the individuals observed in year 1 also could have had a lower growth potential in general.

However, a significant difference between the years also was found in terms of feeding behavior. In year 2, more calves were observed feeding than in year 1, possibly indicating a greater feed intake of the calves observed in year 2. Such a greater feed intake could have led to better weight gain results. Furthermore, the vocalization activity was significantly lower in year 2 than in year 1, potentially indicating that the calves in year 1 were suffering more from weaning stress. Such lower stress tolerance could have been a potential reason for the lower weight gains in this year.

At castration in Trial 3, the WbC calves were 9 days older and about 10 kg heavier than the WsC calves, but these differences were insignificant.
During 3 weeks following castration, the WsC calves, newly weaned at castration, achieved a better growth rate than the WbC calves, which were weaned 8 weeks before castration. This treatment difference possibly resulted from compensatory growth of the WsC calves. Before weaning at almost 210 days of age, the WsC calves potentially experienced a period of low-energy input. This could have been result of deprivation or even lack of milk supply by the dams as well as from an insufficient energy mobilization from solid food. However, LWG, calculated from birth to T0, did not differ between treatments or sexes.

As expected, the castrates had less ADG during 3 weeks after castration than the bulls. This was in accordance to several other studies (Kirkland et al., 2006; Keane and Allen, 1998; Fisher et al., 1996). The decreased weight gain in the recently castrated calves was caused by either castration stress and pain, including swelling of the tissue and inflammatory processes, or the reduction of growth hormones following castration. It could have been also result of the combination of both factors.

During the 3-weeks period following castration, the calves had greater weight gains in year 2 compared to year 1, possibly due to the smaller group size in year 2. As a consequence of housing less animals in the same pen, there was generally more feeding and floor space per animal. These conditions could have been resulting in less competition for food and more floor space usable for lying, respectively. Overall, these factors could be potential reasons for better growing conditions, resulting in the higher weight gains in year 2.
Under the conditions of year 2, the WsC calves achieved ADG during the 3 weeks following castration, that was almost twice as high as that of the WbC calves. However, in year 1, the WsC calves also had a higher growth rate, although the difference between the treatments was not significant. Because the WsC calves had a significant greater ADG in year 2 than in year 1, while the growth rate of the WbC calves did not differ between the years, it could be

presumed that the smaller group size and more space per animal, respectively, was advantageous for the at castration weaned WsC calves.

However, LWG, from birth to castration, was significantly lower for the WsC calves compared to the WbC calves in year 2, whereas in year 1 the treatments did not differ. Furthermore, LWG was significantly different between the years for the WsC, but not for the WbC calves. These differences within the years could not have been due to floor space allowance, as discussed previously for ADG after castration. They may also have been caused by other reasons, such as individual growth potential and the feeding conditions on the pasture during the suckling period as well as indoors for the pre-castration weaned WbC calves. Based on the LWG until castration, it can be assumed that the high ADG during 3 weeks following castration, achieved by the WsC in year 2 (1.64 kg/d), resulted from compensatory growth.

There was no difference between WsC and WbC calves during the pre-pasture period (from 3 weeks after castration to the beginning of pasture period). Both treatments had ADG similar to the growth rate of the WbC calves during the 3-weeks period following castration. These findings also indicated the compensatory growth of the WsC calves previously discussed.

The greater body weight of the WbC calves at the beginning of the pasture period was result of the age of these animals, that were in average 24 days older compared to the WsC calves. This age difference was due to the management. In each year all castrates were taken on the pasture together, while castration was performed on several days, but at similar ages.

Weight gain during the pre-pasture period was almost twice as high for the bulls than for the castrates mainly due to two reasons. One reason was the higher growth potential of intact bulls compared to castrates in general. The second reason was the difference between the feeding regimes of the sexes in this trial. A high-energy fattening ration was fed to the bulls, whereas the castrates got low-energy feed stuff for production management reasons. For the castrates, the feeding of the pre-pasture period was calculated to achieve low-energy input in combination with high fiber content and large volume. Due to this type of feeding, the digestive system of the castrates was prepared to facilitate proper food utilization from the pasture. Castrates` weight gains on the pasture in Trial 3 were in average about 0.79 and 0.83 kg/d for WsC and WbC, respectively (data not shown).

5.5 Slaughter Traits

Treatment did not influence any of the slaughter traits, neither in Trial 2 nor in Trial 3. In Trial 2, animals were slaughtered at a younger age in year 2 compared to year 1, because of a higher LWG from birth to slaughter in year 2. Despite these different slaughter ages, there were no significant differences between the years for the final body weight, the carcass weight and the dressing percentage. Nevertheless the carcasses were classified in a better conformation class and a lower fat class in year 1 than in year 2. The similar final body weights of castrates and intact bulls in year 1 and 2 possibly resulted from the slaughter time in Trial 2, as the decision in terms of slaughter time was made according to the requirements of the market and these did not change fundamentally between the years.

Sex affected all slaughter traits in Trial 2. Bulls were more than 3 weeks older than castrates, when slaughtered. The bulls had a higher LWG till slaughter time compared to the castrates, due to different growth potential of the sexes. Such difference concerning growth potential was also found by other studies (e.g. Kirkland et al., 2006; Keane and Allen, 1998). In combination with the older age at slaughter, this increased growth rate of the bulls resulted in a higher final body weight. Dressing percentage was 2.8% higher for the bulls than for the castrates. This result was in agreement with Temisan (1989), who reported about castrates` carcasses having a 2 to 3% smaller dressing compared to bulls of the same genetic origin. In Trial 2, the carcasses of the castrates were classified closer to the conformation class “R” and in a higher fat class than the bulls` carcasses. However, Kirkland et al. (2006) reported that dressing percentage as well as carcass conformation and carcass fat classification were not significantly different between castrates and intact bulls.

Interestingly, a year x sex interaction was found for slaughter age, final body weight and also carcass weight in Trial 2. This may have been caused by the different genetic origin of the animals in year 1 and 2, which possibly implicated different growth potential and growth performance of castrates and bulls, based on the same fattening feeding system for both sexes. Slaughter age did not differ between castrates and bulls in year 1, but the castrates were slaughtered at a younger age than the bulls in year 2. In both years, final body weight and also carcass weight were lower for the castrates than the bulls. However, in terms of these two slaughter traits, no difference was found for the castrates between the years, whereas the bulls had a higher final body weight and carcass weight in year 2 compared to year 1. These results could have been caused by the purebred Aubracs fattened in year 2. Aubrac bulls were found to have the highest increase of live weight in a study on different genotypes (Junka et al., 2010). Besides purebred Aubracs, five other purebred genotypes (Limousin, Charolais, Simmental, Lithuanian Red and Lithuanian Black-and-White) and also two crossbred genotypes (Lithuanian Black-and-White x Limousin and Lithuanian Red x Limousin) were included. Average live weight gain was 1.28 kg/d in Aubracs, while Limousin and Lithuanian Black-and-White x Limousin achieved 0.90 and 1.15 kg/d, respectively.

In Trial 3, depending on the different fattening systems applied on castrates and intact bulls, an effect of sex was found on each of the slaughter traits. Fattening of castrates included a pasture period for the yearlings, whereas bulls were intensively fattened by feeding a high-energy diet. Therefore, at slaughter, the castrates were about 7.5 months older than the bulls. Due to the older slaughter age, castrates had a greater BW at slaughter than the bulls.

LWG, calculated from birth to slaughter, was lower for castrates than bulls in Trial 3, possibly due to two main reasons. The first potential reason was the difference between the feeding systems during the fattening period, as mentioned before, while the second reason potentially was the lower growth potential of castrated compared to intact male cattle. Temisan (1989) reported for castrates up to 30% lower growth rates than for bulls of the same breed, depending on physiology as well as on feeding strategy.

6 CONCLUSIONS

In terms of weaning distress, displayed by vocalization activity, the older weaning age should be preferred. However, castration seemed to reduce vocalization activity. Therefore, the vocal response of the calves did not conclusively indicate their well-being status, when weaning and castration occurred simultaneously. Based on the different vocalization activity of male and female calves, further research should be conducted, how weaning management strategies could be adapted to calf sex. As vocalization activity seems intensely influenced by individuality as well as group dynamics, (field) studies with regard to vocalization should include replications.

In regard to calf behavior, also weaning at an older age should be favored. As behavior was influenced by castration and weaning as well as by housing conditions, studies should be conducted in respect of the physical environment of the animals. Further observations should also differentiate the types of behavior and be made with concern to the individual animal.

Haptoglobin did not indicate the stressors weaning and castration under the conditions of this study. In general, a modulated time schema for blood sampling, closer to the stressful events (castration / weaning) should be performed in future studies. However, blood sampling for analyzing stress, that is close to the occurrence of the stressor, is difficult to combine with behavioral observations.

With reference to a short-time growth performance up to three weeks following the stressor, weaning at a younger age as well as the simultaneous performing of weaning and castration had no disadvantages but rather were favorable. However, in regard to the overall weight gain weaning at an older age is preferable. Performance differences due to timing of castration and weaning were weakened throughout the fattening period. Therefore, at slaughter, only differences between castrates and bulls occurred.

Recommendations, especially for steer producers concerning the timing of weaning and Burdizzo castration, have to be differentiated for the individual production conditions, such as housing, feeding and marketing management.

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