A Single Dose of Ketoprofen in the Immediate Postpartum Period has the Potential to Improve Dairy Calf Welfare in the First 48 hours of Life

Highlights

- Behavioural differences were observed in calves treated with ketoprofen compared to controls for up to 48 h postpartum
- No time/treatment interaction effect was observed, suggesting the analgesic effect of ketoprofen lasted for up to 48 h
- Behaviour changes observed following ketoprofen administration suggest a positive welfare benefit for all calves
- Behaviours of very young calves are described, adding to the current knowledge base where few data are currently available

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Abstract

The welfare impact of birth on newborn calves has rarely been studied. Dystocia in particular may have significant welfare costs for calves. While analgesia is sometimes provided to calves born to difficult parturition by veterinary surgeons in practice, it is not known if this is actually beneficial. On a commercial dairy farm, we examined the behavioural time budget of 39 Holstein heifer calves born with the aid of farmer assistance and 36 calves born without assistance; half of each group were randomly allocated to receive either a single dose of the non-steroidal anti-inflammatory drug ketoprofen or a saline placebo in a two by two factorial design. The calves were group housed and their behaviour was recorded for 48 hours postpartum and analysed using instantaneous focal sampling (every 5 minutes in alternate hours). Regardless of analgesic treatment, calves born with assistance showed behaviours consistent with experiencing a less positive welfare state (lying with their head down and in lateral recumbency, and less time playing than unassisted calves). Behavioural differences between calves treated with ketoprofen and calves treated with saline (in particular increased play) suggest that the birth experience may be painful for all calves, even if no assistance is required. Our findings suggest that a single dose of ketoprofen in the immediate postpartum period may improve calf welfare regardless of assistance status and has the potential to contribute to significant welfare gains in dairy calves.

Key Words: Calf, welfare, behaviour, pain, parturition, analgesia

1. Introduction

Parturition is regarded as a painful and stressful event by both farmers and veterinary surgeons (Laven et al., 2009; Remnant et al., 2017; Whay and Huxley, 2005). Given that rates of assistance in calving have been reported to range from 10% to 50% in dairy cows (Mee, 2008), pain during and following dystocia has the potential to have a significant welfare impact on both the calf and the dam. However, there is a paucity of research in this area, particularly regarding the pain experienced during birth and the welfare impact of dystocia on the calf (Laven et al., 2012; Murray and Leslie, 2013). A difficult calving has been shown to adversely affect the vigour of the neonatal calf as assessed using criteria such as modified Apgar scores, behavioural analysis in the immediate postpartum period and clinicopathological measurements (Adams et al., 1995; Barrier et al., 2012; Murray and Leslie, 2013; Riley et al., 2004), with similar findings being reported in other domestic ruminant species (Dwyer, 2003; Dwyer et al., 1996). While calves experiencing dystocia are not reported to differ from calves born to a normal calving in terms of number of attempts to suckle the dam, they have been shown to be less likely to achieve successful suckling within three hours of birth than unassisted counterparts (Barrier et al., 2012). Successful suckling in this instance was defined as the teat of the dam being observed to be in the calf's mouth for more than five seconds with calf positioned under the udder (Barrier et al., 2012). A Canadian study showed that while an assisted calving was not associated with a reduced intake of colostrum per se, reduced vigour in the first hour of life was associated with reduced colostrum intake (Vasseur et al., 2009).

It is possible that differences in immediate postpartum behaviour and vigour in calves experiencing dystocia may result from pain secondary to tissue trauma caused during parturition (Mee, 2013); however, there may also be other factors involved, such as exhaustion. Currently, there is a lack of knowledge of the effects of dystocia on behaviours not directly linked with vigour over the first days (as opposed to hours) of life. It is possible that delayed behavioural responses may be exhibited in calves related to the effects of dystocia and assisted parturition, although it has been demonstrated that assisted parturition is not associated with delayed expression of maternal behaviour in cows (Barrier et al., 2012).

Pain is difficult to assess as it cannot be measured directly, but behaviour is the parameter most often used to assess animal pain (Molony and Kent, 1997; Rutherford, 2002; Weary et al., 2006). Intervention with analgesics is a widely used approach in animal welfare research, because it is generally agreed that the abolition of suspected pain-related behaviour with analgesic is circumstantial evidence of pain (Rutherford, 2002; Walker et al., 2011). Thus, differences in the behaviour of calves experiencing dystocia that are given pain relief, compared to those given a placebo, may indicate that these effects are pain related. However, care must be taken with the choice of agent and the dose administered since analgesic drugs may have behavioural effects unrelated to pain and nociception, and some also have physiological side effects. Additionally, the choice and dose of agent provided to production animals is often limited by regulations regarding the use of drugs licensed for use in food producing animals such as dairy and beef cattle. In the UK, most analgesic agents licensed for use in food producing species are non-steroidal anti-inflammatory drugs (NSAIDs) (National Office of Animal Health, 2017). NSAIDs are commonly used in farm animal veterinary practice for their antipyretic, anti-inflammatory and analgesic properties and there are a number of licensed formulations available for use in cattle as well as other farmed species (Lees et al., 2004; Whay and Huxley, 2005).

The objective of this study was to assess pain associated with assisted birth by investigating whether the behaviour of calves experiencing both assisted and unassisted birth on a commercial dairy farm was affected by the administration of analgesia immediately after birth. Our hypothesis was that administration of a single dose of NSAID analgesia in the immediate postpartum period to calves subject to assisted birth would be associated with differences in the behavioural time budget over the first 48 hours of life, compared to calves administered a placebo or subject to unassisted birth. As pain is a source of poor welfare, we surmised that any differences in behaviour indicative of reduced pain would also indicate an improved welfare state in these animals. The two by two design of this study (assisted/unassisted and analgesic/placebo) allowed us to determine both the effect of calving assistance on neonatal calf behaviour and the effect of administration of analgesic. Ketoprofen was selected for this study; it is a propionic acid NSAID that is commercially available as a 50:50 racemic mixture of two enantiomers (R(-) and S(+)) (Landoni et al., 1995; Stock and Coetzee, 2015). In calves, ketoprofen has been shown to have rapid clearance from plasma (within three hours); however, clearance from inflammatory exudate is much slower (twelve hours for complete clearance) (Landoni et al., 1995).

The experimental design involved randomly allocating forty-nine cow-calf pairs receiving farmer assistance at parturition and forty-seven time matched cow-calf pairs not receiving any assistance to either a treatment group or a placebo group. We focused on mild and moderate farmer-provided assistance because previous work has indicated that farmers are less likely to provide analgesia following an assisted calving than veterinary surgeons (Huxley and Whay, 2007; Remnant et al., 2017; Whay and Huxley, 2005). Here, behavioural results for calves are reported; postpartum behaviour of cows and biochemical parameters and production measures for both cows and calves were also monitored and are reported separately (Gladden et al., in prep; Gladden et al., 2018).

2. Materials and Methods

2.1 Farm management and husbandry

A 700 cow Holstein dairy herd in Scotland, UK was recruited to take part in the study. The herd is housed all year round and approximately 60 cows calve each month throughout the year. Lactating cows and far-off dry cows (cows three to eight weeks pre-partum) are housed in cubicles; three weeks prior to expected parturition, cows are moved to a straw-bedded group calving pen. Cows calve in this pen and are moved to an adjacent postpartum pen at, or before, the next milking after parturition. Cows are milked three times daily at eight hour intervals, therefore the maximum amount of time a cow might spend in the calving pen after calving is eight hours. Animals are fed a grass silage based total mixed ration (TMR) ad libitum and have ad libitum access to water. Calves are removed from the dam as soon as is reasonably possible after birth and moved to a straw-bedded group calf pen measuring 3.1m x 2.9m. On average, 4 to 6 calves occupy each calf pen at any one time. All calves are fed a minimum of 4.5 litres of colostrum within four hours of birth. Colostrum is initially offered from a bottle; if calves do not suckle well, or do not drink the full 4.5 litres, colostrum is fed via an oesophageal feeder to ensure every calf has prompt, adequate colostrum intake. Calves that drink the full 4.5 litres enthusiastically are offered more colostrum from a bottle and are allowed to drink more colostrum as required. Each calf is weighed and has the umbilicus dipped with a 7.5% iodine solution when they are moved to the calf pens. Calves are fitted with an approved ear tag within 36 hours of birth in accordance with European legislation regarding identification of cattle (regulation (EC) 911/2004). After moving to the calf pen, the calves are fed 3.5 litres of powdered milk replacer (Provimilk; 21% crude protein, 18% fat) twice daily with a group teat feeder. If calves do not suckle initially, or are pushed away from the feeder by other calves, they are fed individually with a bottle or an oesophageal feeder as deemed necessary by the farmer.

2.2 Study design

A two by two randomised control trial was developed to include cows and calves requiring assistance at parturition and also unassisted controls. Calves were randomly assigned to receive either ketoprofen analgesia or saline placebo; randomisation was performed using a randomly generated Latin square (Pezzullo, 2016) with odd numbers representing placebo and even numbers representing treatment. Animals were designated as either assisted or unassisted parturition, and the level of assistance was graded, by an experienced stockman in accordance with a one to four grading system provided by the authors and modified from Barrier et al. (2013, 2012) [*Table 1*]. Calves born to grade 1 parturition were assigned to the 'unassisted' group and calves born to grade 2 (mild farmer assistance) and grade 3 (moderate to severe farmer assistance) parturition were assigned to the 'assisted' group. When an assisted calf was recruited, the next eligible unassisted calf born was recruited as a time-matched control to account for seasonal effect. Calves born to grade 4 parturition (veterinary assistance or caesarean section) were not eligible to be included in the study. All calves included in the study were female purebred Holstein dairy calves (*Bos taurus*). Recruited calves were not managed differently from other calves in the herd; calves were moved into group pens in line with the routine management on the farm, the assistance or treatment status of the calf did not affect the farm calf management or the choice of pen placement.

Power analysis based on pilot data of calf behavioural monitoring (Strazhnik et al., unpublished) indicated that 40 calves recruited to each assistance/non-assistance group would be sufficient to detect small (3%) changes in time budget. In total, 95 calves were recruited onto the study by the end of the data collection period. Due to equipment failure, incomplete video footage was obtained for some calves and these calves were removed from the study. Seventy-five calves were included in final behavioural analysis; 39 calves experiencing assistance at birth (21 placebo and 18 ketoprofen) and 36 calves not experiencing assistance at birth (16 placebo and 20 ketoprofen). Animal recruitment and data collection took place over a single period between the 7th of March and the 17th of December 2016.

2.3 Animal recruitment and experimental procedure

When animals were determined to meet the inclusion criteria for recruitment the study veterinary surgeon (NG) or the head stockman were notified. In this study, both the cow and the calf received treatment or placebo; administration was performed only by the author (NG) or the head stockman. Cow-calf pairs allocated to the treatment group were injected with ketoprofen (Ketofen 10%, Merial Animal Health Ltd., Essex, UK) at the manufacturer's recommended dose rate of 3mg/kg bodyweight (equivalent to a volume of 1ml/33kg bodyweight) by deep intramuscular injection. Ketoprofen was chosen for use in this study as it is a licensed product with zero milk withdrawal period (UK) for the adult cattle included in the project (National Office of Animal Health, 2017) and, although not licensed, it has been used safely in calves as young as three days old (Merial Animal Health Ltd., 2007). Cow-calf pairs allocated to the placebo group were injected with saline (Vetivex 1, Dechra Veterinary Products, Shrewsbury, UK) by deep intramuscular injection at the same volume dose rate (1ml/33kg bodyweight). Ketoprofen or saline was administered within three hours of parturition, if this was not possible the animal was not included in the study. Sequential jugular blood samples were obtained at the same time as treatment or placebo administration, or shortly afterwards, from calves on the day of parturition and also at 24 hours, 48 hours and 7 days after parturition for biochemical analysis as part of a larger study. All animals were inspected clinically by a veterinary surgeon (NG) when recruited onto the study and also when leaving the study. All calves were clinically healthy when recruited; if clinical abnormalities were identified affected calves were not recruited onto the study. In accordance with Home Office regulations, any clinical abnormalities identified when the calves were signed off the study were recorded; only one of the calves recruited showed any clinical abnormality, this calf presented clinical signs consistent with respiratory disease.

All cows and calves recruited to the study were marked with agricultural spray marker on the lumbosacral region to improve ease of identification on video footage and also to mark these animals as being on the study and therefore subject to UK Home Office regulations. This type of marking is not used on the farm for any other reason. Additionally, all animals were photographed when recruited to assist identification during video analysis.

The identification numbers of the dam and calf, the date and time of calving, the degree of assistance required for calving, the time the calf was moved into a calf pen, the number of the pen, the birth weight of the calf and the amount of colostrum fed to the calf were all recorded by farm staff in a chart provided and positioned next to the calf pens on the farm. Dam lactation number was obtained from herd records. Which product the animal received, and the volume administered was recorded; these data were stored in the farm office for the duration of the study and was not available to the observer during video analysis.

The study was performed under UK Home Office Project and Personal licence authority; after the experiment all animals were returned to the herd after being discharged from the controls of Animals (Scientific Procedures) Act following veterinary inspection.

2.4 Behavioural monitoring and data collection

Closed circuit television (CCTV) cameras (Sony CCD, Vari-focal, 700TVL, Sony, Minato, Tokyo, Japan) were set up to continuously film the calving pen, the postpartum pen, and six neonatal calf pens. To monitor the calf pens one camera per pen was positioned at a height of 2.17m. Cameras were positioned at a height that would not interfere with animal behaviour or farm machinery whilst ensuring adequate picture quality for observational analysis.

Footage was continuously filmed and stored on digital video recorders (DVR) (Guardian II+ DVR 8 Channel, Digital Direct Security, Huntingdon, UK) on farm. The required footage was backed up regularly (minimum twice weekly) onto an external hard drive (Seagate 1TB portable external hard drive, Seagate Technology LLC, Cupertino, USA) for long term storage.

2.5 Behavioural analysis

An ethogram for calf behaviour based on pilot observations was refined to develop the final ethogram [*Table 2*]. Due to time taken for the calf to be moved into an individual pen, behavioural monitoring began two hours post-calving; the time of calving was recorded by farm staff.

Data were analysed using instantaneous sampling (Martin and Bateson, 2009) to establish a behavioural time budget for each calf. A sampling interval of every five minutes every other hour was determined to be appropriate during pilot work (Strazhnik et al., unpublished); behaviour every even hour of the 48 hours postpartum period (i.e. two hours postpartum, four hours postpartum and so on) was recorded. Behavioural observations were performed in four time blocks; 0 - 12 hours postpartum (60 time points, starting at 2 hours postpartum), 12 - 24 hours postpartum (72 time points), 24 - 36 hours postpartum (72 time points) and 36 - 48 hours postpartum (84 time points, including the 48^{th} hour). Thus, a total of 288 time points were recorded for each calf.

The observer was blinded to both treatment and assistance status of each calf. Calves were identified by the spray mark on the lumbosacral region and the identity of the calf was further confirmed prior to starting observations by comparing to the photographs taken at the time the calf was recruited onto the study. Calves were identified by the last four numbers of their UK ear tag number. The behaviour (and posture in the case of lying behaviours) exhibited by the calf at each time point was recorded in a spreadsheet (Excel 2013, Microsoft, Redmond, Washington, USA) in the form of letter codes corresponding to the ethogram [*Table 2*]. Calves that were out of view of the cameras were recorded as 'not visible'.

Primary behaviours (lying behaviours, attempting to stand, standing, walking, play or not visible) were recorded for all time points. Lying behaviour was subdivided into body position (lateral recumbency, sternal recumbency or unknown) and head position (up or down). Calves identified as being in a lying position but where the posture could not be identified were recorded as 'lying unknown'. Active primary behaviours included attempting to stand, standing, walking and play behaviour. Secondary behaviours (grooming behaviours, feeding directed behaviours, social behaviours and miscellaneous behaviours) were exhibited concurrently with primary behaviours (for example, a calf would be grooming itself whilst either in a standing or lying position) and were recorded whenever they occurred.

2.6 Statistical analysis

All data were summarised in Microsoft Excel (2013, Redmond, Washington, USA). For every calf each behavioural observation recorded was counted in Excel and a time budget was calculated for each behaviour. Time budgets for both the total time (number of times a behaviour was observed divided by total number of available time points), and the total proportion of visible time (number of times a behaviour was observed divided by the number of time points the subject was visible) were produced.

All behaviour data were analysed using Genstat (14^{th} Edition). Statistical significance was based on F statistics and $p<0.05$ threshold level. Statistical comparisons of behavioural variables were conducted via Generalised Linear Mixed Models (GLMM) (Poisson distribution) or Linear Mixed Models (LLM) (normal distribution) dependent on the data distributions for each variable. Data transformations were attempted when necessary via Logarithm function. All models included calf identity (ID) as a random effect. All fixed effects were treated as factors and all interactions between factors were included in maximal models. All minimal models included treatment (i.e. ketoprofen or placebo treatment), assisted/unassisted parturition, time period and the interactions between treatment and assisted/unassisted and treatment and time period. Co-variates included in the models included the median number of calves in pen, birthweight, and dam lactation number. Dispersion was fixed dependent on the variable. Correlations between variables and fixed effects were performed as Pearson's Correlations for parametric data, and Spearman's Rank Correlations for non-transformable non-parametric data.

Due to the small proportion of time engaged in non-lying behaviours (i.e. 'active time'), some behaviours (play, walking, standing and attempting to stand) were analysed both as absolute proportions and also as a proportion of active time. All behaviours were analysed as the proportion of the time the calf was observable.

3. Results

Seventy-five calves were included in the final behavioural analysis. Mean calf birthweight was 42.8kg (range 27.6kg to 62.8kg) and 49% of the calves recruited were born to primiparous dams; this reflects a combined effect of herd parity distribution and the use of sexed semen in primiparous animals. Assistance status was not affected by calf birthweight or dam parity. The median number of calves in the pen did not have an effect any of the behaviours studied.

Lying was the most frequently observed behaviour for all calves which accounted for more than 80% of the time budget in all groups. Lying in sternal recumbency was overall more common than lying in lateral recumbency. The most common non-lying behaviour observed was standing, which accounted for 10% of the overall total time budget. Standing accounted for more than 70% of the overall active (non-lying) time. Secondary behaviours were not observed at all time points and were only observed 5% of the time. The most common secondary behaviour observed was investigatory behaviour (1.8% of time). Overall, other secondary behaviours were observed on average 1% of time or less [*Table 3*]. The least common behaviours and postures observed were lateral recumbency with unknown head position, lying in unknown positions, grooming others and other secondary behaviours. All of these accounted for less than 0.5% of the time budget for all groups and are not further presented.

Over the whole 48 hour observation period, the mean amount of time calves could not be observed was 4.6%. The proportion of time for which calves could not be observed differed between observation periods; not visible was recorded most in the 0 to 12 hour time period (mean 14.7%; p<0.001).

3.1 Effects of assistance status

Regardless of treatment status, across all time periods, calves born to assisted parturition were observed lying with their head in a down (rested) posture more (*p*=0.008), and their head in an up (alert) posture less than (*p*=0.038), than calves born to unassisted parturition. Calves born to assisted parturition showed a tendency to lie in lateral recumbency more than those born to unassisted parturition (*p*=0.080). Assisted calves also engaged in investigatory behaviours more often than calves born to unassisted parturition (*p*=0.036) [*Table 4*]. Calves born to unassisted parturition engaged in play behaviour more than calves born to assisted parturition (*p*=0.019) [*Table 4*].

3.2 Effects of treatment

Regardless of assistance status, calves administered saline placebo showed a tendency to spend more time lying in lateral recumbency than calves administered ketoprofen (*p*=0.052) [*Table 5*]. Calves in the placebo group showed a tendency towards spending more time with head in a down posture (*p*=0.078) [*Table 5*]. Calves in the ketoprofen treated group spent a greater proportion of the total time budget engaging in a range of secondary behaviours (such as social behaviours, feeding behaviours and grooming behaviours) (*p*=0.011) [*Figure 1*] and play behaviour (*p*=0.017) [*Figure 2*]. Calves in the ketoprofen treated group spent more time engaged in self-grooming behaviour than calves in the placebo group (*p*<0.001). Social grooming behaviours were unaffected by analgesic treatment.

3.3 Effects of assistance and treatment interaction

There was an effect of interaction between assistance and treatment on walking behaviour; calves born to assisted parturition that were treated with ketoprofen engaged in walking behaviour more than calves in any of the other three groups (*p*=0.004) [*Figure 3*]. Calves born to unassisted parturition that were treated with ketoprofen analgesia spent less time (2.70% of time) in lateral recumbency than calves in any of the other three groups (compared to 7.37% of time in the assisted treatment group, 7.81% in the assisted placebo group and 8.03% in the unassisted placebo group) (*p*=0.005). Additionally, calves in the unassisted ketoprofen group tended to spend more time in sternal recumbency than calves in any of the other groups (*p*=0.056).

Assistance/treatment interaction did not have an effect on play behaviour as a proportion of the total time budget; however, when play behaviour was analysed as a proportion of active (i.e. non-lying) time, there was a tendency for ketoprofen treated calves born to unassisted calving to spend more of their active time engaged in play (*p*=0.062).

3.4 Effects of time period

The 48 hour time budget was divided into four 12 hour time periods for analysis (0 to 12 hours, 12 to 24 hours, 24 to 36 hours and 36 to 48 hours). Regardless of treatment or assistance status, calves spent most time engaged in play behaviour in the 12 to 24 hour time period (1.49 %) and the amount of time engaged in play declined from this peak as time progressed (*p*=0.001). When play was analysed as a proportion of active time (rather than total time), the same effect was observed with increased significance (*p*<0.001) [*Figure 4*]. There was a tendency for all calves (irrespective of assistance or treatment status) to spend less time with their head in a down (rested) position as time progressed up to 36 hours postpartum (*p*=0.069).

The total proportion of time that calves exhibited secondary behaviours (feeding directed behaviours, grooming behaviours, investigatory behaviours and social behaviours) increased from 4.23% in the 0 to 12 hour time period up to 6.84% in the 24 to 36 hour time period (*p*<0.001). Time spent exhibiting each individual secondary behaviour was not affected by time with the exception of self-grooming behaviour which increased from 0.38% in the 0 to 12 hour time period to 1.57% in the 24 to 36 hour time period (*p*<0.001). A tendency for feeding behaviour to increase with time was observed (from 0.70% in 0 to 12 hour period up to 1.34% in the 24 to 36 hour time period) (*p*=0.06).

Calves spent less time attempting to stand as time progressed (*p*<0.001). During the 0 to 12 hour time period, calves were observed to be attempting to stand 14.4 times more than in the 36 to 48 hour time period.

No effect of time/treatment interaction was identified for any of the behaviours analysed.

3.5 Effects of other factors

Irrespective of assistance or treatment status, calves born to dams in lactation four or over spent less time in sternal recumbency than calves born to younger dams (*p*=0.002), more time in lateral recumbency (*p*=0.014) and more time engaged in active behaviours (*p*=0.047). Dam lactation also affected time engaged in overall secondary behaviours and investigatory behaviours with calves born to older dams spending more time engaged in these behaviours (*p*=0.007 and *p*=0.007 respectively).

An increasing number of calves in the pen was associated with an increased proportion of time calves could not be observed (*p*=0.011). The number of calves in the pen also had an effect on calf head position, with calves spending less time in a head down position as median number of calves in the pen increased (*p*=0.045). Calf birthweight did not have an effect on any lying behaviours but did affect head position. Heavier calves spent more time with their head held up in an alert position (*p*=0.023) and less time with their head in a down (rested) position (*p*=0.029). Walking was also affected by calf birthweight; heavier calves spent more time engaged in walking behaviours. Lighter calves engaged in more self-grooming and more combined secondary behaviours than heavier calves (*p*=0.022 and *p*=0.01 respectively).

4. Discussion

The novel randomised design of this study allowed us to examine in detail whether neonatal calves born to assisted parturition exhibit behavioural differences suggestive of pain and whether these are ameliorated by the administration of NSAID analgesia in the immediate postpartum period. As hypothesised, the study found behavioural differences such as reduced play in calves born with assistance. There was some evidence of reversal of these effects with analgesic treatment, with effect of interactions between assistance and treatment for some behaviours, such as lateral recumbency. These results suggest that birth may be an uncomfortable experience for all calves and that the welfare impact of assistance at birth is greater still. Previous studies have demonstrated that assisted parturition has a negative effect on the vigour of newborn calves in the immediate postpartum period but prior to this study, there was little evidence to indicate whether providing analgesia to neonatal calves at birth is beneficial, despite evidence suggesting that this is sometimes done in clinical practice (Laven et al., 2012; Remnant et al., 2017). Additionally, it was also unknown whether any adverse effects experienced by calves during birth persist beyond 24 hours, or whether calves experience delayed onset pain that is not immediately apparent at the time of birth.

An unavoidable limiting factor in any study of this design is the proportion of time neonatal calves spend lying and the small remaining proportion of the time budget devoted to active and luxury behaviours (e.g. play). In this study, lying behaviours predominated as expected, with more than 80% of the total time budget spent lying in all time periods; this is normal behaviour for neonatal calves (Chua et al., 2002; Hill et al., 2013). To improve precision, active behaviour was considered as a proportion of active time (i.e. time not spent lying) as well as a proportion of total time; however, no significant differences were observed. Another limiting factor difficult to avoid in observation studies is the inability to observe animals at some times, although this accounted for only 4% of total observations here. In this study, calves were out of view most in the 0 to 12 hour period; 42 calves were not moved to the neonatal calf pens in the first two hours postpartum, although most of these had been moved into a calf pen by the end of the second observed hour (i.e. by five hours postpartum). Preliminary data obtained prior to this study indicated that most calves were moved within three hours of birth, so the high number of calves not moved to the neonatal pens before behavioural observations began was unexpected. Some very early neonatal behaviours will have been missed as a result; however, there was no association between either assistance or treatment status and time out of view. In the other time periods, the most common reason for calves not being observed was due to the position of the calf in relation to the camera; if a calf was positioned directly underneath the camera, it could not be seen. This is the likely reason for the number of calves in the pen affecting the proportion of time calves were recorded as 'not visible' as when the pens were more full, calves occupied more of the pen perimeter and were observed lying in the corners more often. When there were few calves in the pen, it was noted that calves chose to lie in the centre of the pen rather than at the edges (data not presented). Instantaneous scan sampling was used in this study due to the duration of the postpartum period of interest (48 hours), rendering continuous observation impractical. Although pilot work informed the sampling period used, this method of sampling has limitations, especially with regard to infrequent or sporadic behaviours which can be missed. This results in the amount of time engaged in certain behaviours to be underestimated. However, the behaviour observation technique was the same for all calves, and the observer was blinded to both assistance and treatment status, so any inaccuracies are likely to have affected all calves equally. The fact that significant effects on sporadic behaviours such as play were still identified using instantaneous scan sampling suggests that the effects identified are robust. Technological advances in remote behaviour monitoring (via accelerometery data from a sensor worn by the animal), validated against behavioural observations, may enable accurate capture of sporadic behaviours and we are exploring this for future work.

In a study such as this on a commercial farm, whilst the assistance status was determined by referring to a well described scoring system, the decision to intervene and assist at parturition was made by the farmer. A study of 249 farms in Ireland indicated that parturition is assisted by farmers in up to 48% of heifers and 35% of cows; however, the authors suggested that many of these animals may have gone on to calve unaided if given enough time (Egan et al., 2001). On the farm studied here, assistance at parturition (and the decision to intervene) was primarily performed by one experienced person, meaning that assistance provided was skilled and consistently performed in most cases. Animals are closely monitored for parturition, and early intervention to assist parturition on the farm was not performed unless the calf was malpresented; an approach that has been suggested to provide the optimum balance between reducing the risk of stillbirth (early delivery) and reducing the risk of trauma to the cow (allowing time for soft tissue relaxation and dilation) (Schuenemann et al., 2011). A second experienced stockman provided parturition assistance on occasions where the primary stockman was unavailable (for example holidays) which is reflective of commercial practices on UK cattle farms.

Ketoprofen is rapidly absorbed and clinical effects start to be observed soon after administration (Landoni et al., 1995; Whay et al., 2005). In this study, treatment was administered to all calves within three hours of birth and behavioural observations began at two hours postpartum. Due to the rapid onset of action of ketoprofen, it is therefore unlikely that behavioural observations of many calves commenced prior to onset of action of ketoprofen. In cases where this may have occurred, or in cases where ketoprofen was administered after behavioural observations began (i.e. between two and three hours postpartum), this is unlikely to have affected the overall findings as the maximum amount of time that may have elapsed between starting behavioural observation and administering ketoprofen was one hour, which accounts for a small proportion of sampling points. Ketoprofen is not licensed in the UK for use in calves under six weeks old, however the product has been used safely in calves as young as three days old (Merial Animal Health Ltd., 2007). As such, the use of ketoprofen in newborn calves is off licence (but does comply with the prescribing cascade [Veterinary Medicines Directorate, 2015]) and is at the discretion of the attendant veterinary surgeon. Although gastrointestinal ulceration is known to be an adverse side effect of NSAID administration in other species, a direct correlation between NSAID use and abomasal ulceration in cattle has yet to be proven (Hund and Wittek, 2018; Walsh et al., 2016) and to our knowledge, NSAID side effects reported in other species (such as blood dyscrasias and renal failure) have not been reported in cattle. Additionally, adverse side effects of NSAID administration are usually reported to occur in animals that are administered NSAIDs for a long duration, at high doses, or in conjunction with other drugs that may have contributed to the observed effects (Lascelles et al., 2005; Luna et al., 2007), being rare following a single dose at the recommended dose rate. Few data are available regarding the pharmacokinetics of ketoprofen in such young neonates, although altered pharmacokinetics are reported in young children compared to adults (Litalien and Jacqz-Aigrain, 2001). Wilcke et al. (1998) demonstrated altered ketoprofen pharmacokinetics in foals less than 24 hours old (compared to adult horses), and concluded that the dose rate of ketoprofen in young foals should be increased to account for this (Wilcke et al., 1998). Although possible, it is unlikely that a single injection of ketoprofen (at the recommended dose rate) would produce adverse side effects in healthy calves. An additional consideration in newborn animals is the possibility that NSAID administration might affect renal development and function. Whilst many neonatal animals have immature renal function, this has not been found to be the case in calves (Dalton, 1968). The effect of NSAIDs on bovine renal development has not been studied, but in mice only cyclo-oxygenase (COX) -2 selective inhibitors have been shown to impede normal renal development, whereas COX-1 inhibition has not been shown to have any effect (Hörl, 2010). As ketoprofen is a non-selective COX-inhibitor (and given the relative maturity of the neonatal bovine kidney) the risk of impaired renal development or function in calves on this study was considered to be low. All calves recruited to the study currently remain in the herd and no unexpected illness or other abnormal signs that might be consistent with poor renal development or renal dysfunction (for example poor growth rates (Philbey et al., 2009)) have been identified. The expected duration of clinical effect of ketoprofen is up to 24 hours (Landoni et al., 1995); therefore, it might be expected that after 24 hours, the efficacy of ketoprofen might be reduced. In this study however, there was no interaction effect between treatment and time which suggests that the clinical efficacy of ketoprofen possibly exceeds 24 hours in calves. There is some evidence in older cattle that the duration of clinical effect of ketoprofen is longer than the expected duration of action (Whay et al., 2005), and this phenomenon has also been reported in humans (Kantor, 1986). The mechanism behind this however is currently uncertain but may be related to delayed clearance from inflamed tissues (Kantor, 1986).

Lateral recumbency is considered an abnormal lying position in calves (Molony et al., 1995) and is often adopted by calves that are sick or considered to be in pain. Similar to a previous study by Barrier et al. (2012), calves born to assisted parturition in this study tended to spend more time in lateral recumbency. This study monitored calf behaviour for longer than Barrier et al. (2012) and there was no effect of time; suggesting that pain and discomfort following birth in calves born to assistance may be experienced beyond the first three hours (as demonstrated by Barrier et al., 2012) and possibly for as long as 48 hours postpartum. When assistance groups were combined, analgesic treatment alone tended to have a positive impact on the amount of time spent lying in lateral recumbency; calves in the ketoprofen group spent almost half as much time in lateral recumbency than calves in the saline placebo group, supporting the hypothesis that calves in pain were more likely to adopt a position of lateral recumbency. Unlike lateral recumbency, sternal recumbency was not affected by either treatment or assistance status, although there was a tendency for calves born to unassisted parturition and treated with ketoprofen to spend more time in sternal recumbency than calves in the other groups. Sternal recumbency was however affected by dam lactation; calves born to younger dams spent more time lying in sternal recumbency than calves born to dams in lactation four and older. In this study, 83% of calves were born to dams in lactations one to three (inclusive) and, although this is reflective of the farm herd profile, it is a limiting factor for the study because calves cannot be matched for dam lactation. Overall, sternal recumbency was the most common individual behaviour observed in all groups and it is possible that sternal recumbency was observed more often in calves born to younger dams because there were more calves in the study born to younger dams.

The purpose of play is not fully understood (Held and Špinka, 2011; Martin and Caro, 1985), although it is widely considered to be an indicator of positive welfare (Held and Špinka, 2011; Jensen et al., 1998). As such, play is considered to be a 'luxury' behaviour that is exhibited in the absence of negative welfare states. We identified an effect of time on play behaviour; calves engaged in play most in the 12 to 24 hour time period and play behaviour reduced from then onwards. Few previous studies have analysed play behaviour in calves as young as those reported here so it is difficult to determine whether this is a normal pattern of behaviour over time in very young calves. We identified significant differences between calves treated with saline placebo and calves treated with ketoprofen, suggestive of improved welfare in the calves receiving analgesia. Although calves born to assistance engaged in play behaviour less often than calves experiencing unassisted birth, the interaction between assistance and treatment was not significant. This may suggest that assistance is of lesser importance with regard to effect on play behaviour and that provision of analgesia improves the welfare of all neonatal calves, regardless of assistance provided at birth, although it should be borne in mind that assistance on the study farm was probably optimally timed and managed and therefore this effect may not be applicable to all farms.

Calves in the assisted NSAID treatment group engaged in walking behaviours for a greater proportion of time than calves in any other group. It is worth noting that the calves studied here were not placed in situations where walking was necessary or initiated by human contact, therefore walking was voluntary. Similar to other active behaviours, voluntary walking is more likely to be exhibited by calves that are not in pain. Both assisted and unassisted birth has been demonstrated to result in increased serum creatine kinase concentration in calves in the immediate postpartum period (Anderson et al., 1976; Knowles et al., 2000; Murray et al., 2015) and more severe assistance has been associated with higher concentration (Murray et al., 2015). As creatine kinase is a muscle specific enzyme, elevated serum concentrations of this enzyme in cattle are considered an indicator of muscle damage (Anderson et al., 1976; Russell and Roussel, 2007), which is likely to be a source of pain and discomfort. Ketoprofen has been demonstrated to remain in inflammatory tissue for up to 12 hours, and inhibit tissue prostaglandin for up to 24 hours, following intravenous injection in calves (Landoni et al., 1995) and is considered to have a clinical duration of 24 hours (Papich, 2016). The observed differences in walking behaviour provide further evidence that the administration of ketoprofen analgesia to calves experiencing assistance at birth has a positive welfare impact.

With the exception of feeding directed behaviours, the secondary behaviours analysed were ‘extra’ behaviours not required for survival, such as social behaviours. Self-grooming was affected by treatment regardless of assistance status. Molony and Kent (1997) suggested that animals experiencing pain avoid unnecessary movement; therefore, as self-grooming requires movements that include stretching and twisting to reach areas of the body being groomed, it is possible that calves treated with analgesia engaged in more self-grooming due to reduction of pain. Assistance status affected investigatory behaviours, but treatment status or interaction did not. It is possible that pain was a factor involved; however, given the lack of treatment effect, other factors not assessed by this study such as exhaustion may also be affecting investigatory behaviours. Furthermore, investigatory behaviours were often observed to occur at the same time as walking, a behaviour in this study affected by both assistance and treatment, which needs to be taken into account when interpreting effect on investigatory behaviours. Exhaustion per se is difficult to assess in neonatal calves although reduced vigour has been associated with prolonged birth as well as difficult or traumatic birth (Bleul and Götz, 2013; Homerosky et al., 2017). This is related to the presence of hypoxia and associated metabolic acidosis in calves born to birth of prolonged duration which may exacerbate respiratory muscle fatigue (Haddad and Mellins, 1984), leading to exhaustion. The reported study was designed with the objective of investigating pain experienced by calves at birth and any effect of providing analgesia to calves in the immediate postpartum period and calf vigour was not scored due to predicted difficulties in achieving this (similar to those described by

Homerosky et al. (2017)). As such, exhaustion itself has not been assessed in the study reported; however, effects of assistance that were not ameliorated by analgesic treatment were seen, suggesting that pain is not the only factor affecting neonatal behaviour in calves.

The proportion of time spent engaging in feeding behaviour showed a tendency to increase over time. The time available for feeding is controlled by farm management; feeding occurs at set times twice a day and farm staff assist weaker calves to ensure that all calves ingest enough milk. It is probable that farm management had an impact on feeding directed behaviours in this study and this needs to be taken into account when interpreting results. Additionally, due to the design of this study (alternate hours observation), not all feeding times were observed. This is a further limitation to interpretation of feeding directed behaviours in this study and continuous observation of feeding behaviours in an ad libitum feeding system would enable this effect to be explored further.

Occurrence of attempting to stand was affected by time. In this study attempting to stand was defined as adopting a position between lying and standing and was indicative of difficulty in transitioning from a lying position to a standing position. Calves younger than 12 hours old exhibited this behaviour the most and calves older than this were observed to be attempting to stand much less often. This was unaffected by assistance or treatment and likely represents normal calf development and age-related improvement in strength, which has been demonstrated to increase linearly with age following birth in cattle (Nishimura et al., 1996).

5. Conclusion

Calves born to assisted parturition showed behavioural differences suggestive of increased pain and discomfort compared to calves born to unassisted parturition. Differences in time spent engaging in walking behaviour and time spent in lateral recumbency indicate that provision of analgesia ameliorates the painful effect of assistance in these calves. However, differences were also seen between calves in the ketoprofen and placebo groups when assisted and unassisted calves were grouped together. This suggests that provision of analgesia to neonatal calves shortly after birth is likely to result in reduced pain and improved welfare in all calves, regardless of whether they are born with assistance or not. NSAIDs are affordable and readily available to veterinary surgeons and farmers for administration to production animals in the UK and other countries. This study indicates that administration of a single dose of ketoprofen analgesia within the first few hours of life has the potential to have positive impact on the welfare of dairy calves. These results indicate that administration of ketoprofen to the calf should be particularly considered in cases where assistance is provided at birth as some of our findings suggest they may experience enhanced benefits. Potential welfare improvements related to administration of ketoprofen to cows following assisted and normal parturition is also of interest and has been assessed by the authors as part of a wider study; these results will be published elsewhere.

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Tables

Table 1: Calving assistance grading system modified from Barrier et al. (2012, 2013)

Table 2: Ethogram of calf behaviours

Table 3: Overall summary statistics (mean and standard error) of untransformed data for each group (data presented as a proportion of time budget [%]). **AP** = assisted placebo, **AT** = assisted treatment, **UP** = unassisted placebo, **UT**= unassisted treatment. Overall proportion of time engaged in lateral recumbency (head position unknown), unknown lying positions, grooming others and other secondary behaviours was less than 0.5% for all groups and data are not presented.

Table 4: Back-transformed mean and standard error and statistical differences (*p*-value and F-statistic) of proportion of time budget (%) engaged in different behaviours for calves born to assisted and unassisted parturition, irrespective of treatment.

Table 5: Back-transformed mean and standard error and statistical differences (*p*-value and F-statistic) of proportion of time budget (%) engaged in different behaviours for calves in each treatment group (ketoprofen/placebo), irrespective of assistance status.

Figure captions

Figure 1: Mean (± SE) percentage of total time budget engaged in combined secondary behaviours for each treatment (ketoprofen/placebo) irrespective of assistance status. Different letters indicate significant differences between treatments (p=0.011).

Figure 2: Mean (± SE) percentage of total time budget engaged in play behaviour for each treatment (ketoprofen/placebo) irrespective of assistance status. Different letters indicate significant differences between treatments (p=0.017).

Figure 3: Mean (± SE) percentage of total time budget engaged in walking behaviour for each treatment group (ketoprofen/placebo) by assistance status. Different letters indicate significant differences between groups (p=0.004).

Figure 4: Mean (± SE) percentage of active (non-lying) time budget engaged in play behaviour for each time period, irrespective of treatment or assistance status (n=75). Different letters indicate significant differences (p<0.001).

Table 1: Calving assistance grading system modified from Barrier et al. (2012,2013)

Grade	Description	Category
1	No assistance required	Unassisted
2	Mild assistance. No repositioning of the calf or mechanical calving aids required.	Assisted
3	Moderate-severe assistance. Calf is malpresented and requires repositioning and/or mechanical calving aid required to deliver calf.	Assisted
4	Veterinary assistance or caesarean section required	Not eligible for inclusion on study

Table 2: Ethogram of calf behaviours

Category	Behaviour	Description	Key
Lying Behaviours	**Sternal**	Calf is lying on sternum. Each forelimb may be positioned on each side of the body, or the forelimbs may be tucked under the sternum, or both forelimbs maybe on the same side of the body however they are flexed and not extended out to the side. This is subdivided into head up [**U**] (no part of the head is in contact with the ground or body) or down [**H**] (any part of the head/face is in contact with the ground or body) in position. If the body position can be identified but the head position cannot be determined this is recorded as unknown [**K**]	**NU/NH/NK**
	Lateral	Calf is lying on its side with both forelimbs positioned to the same side of the body. This can be either left or right. Head can be up or down. The opposing shoulder is in contact with the ground. This is subdivided into head up [**U**] (no part of the head is in contact with the ground or body) or down [**H**] (any part of the head/face is in contact with the ground or body) in position. If the body position can be identified but the head position cannot be determined this is recorded as unknown [**K**]	**RU/RH/RK**
	Unknown	Calf can be determined to be lying but the body position cannot be identified from the footage available - for example only part of the calf is visible. If the head is visible and its position can be identified this is recorded as up [**U**] or down [**H**] as previously described. If the head position also cannot be determined this is recorded as unknown [**K**]	**KU/KH/KK**
Active Behaviours	**Standing**	Calf is supported in a standing position by all four limbs and all are extended for a duration of more than three seconds. All four limbs are in contact with the ground and the animal is not moving.	**T**
	Attempting to stand	Calf is in a partially-standing position supported by one, two, or three limbs extended with the remaining limbs flexed (which differentiates this from standing). All four limbs are in contact with the ground. The head may be up or in contact with the ground. The calf is in this position unaided by human interaction. Standing is not achieved within three seconds.	**A**
	Walking	The calf is in a standing position and takes more than two steps. Three out of four feet are on the ground at any one time - this differentiates it from solo play behaviour.	**W**
	Play behaviours	Running, jumping (two or more feet off the ground) and skipping. Play can be solitary or social.	**P**
Secondary Behaviours	**Grooming of self**	Calf is grooming self - licking/nibbling body and/or legs. This can be from a standing or a lying position	**S**
	Grooming others	Calf is grooming another calf - licking/nibbling/suckling body/head/legs or a combination of all three. This can be a standing or lying position.	**O**
	Investigatory behaviours	Calf is investigating surroundings. Sniffing, licking, chewing, rubbing, nuzzling, moving with foot or nose any inanimate object - this will include water/feed containers and bars of pen.	**I**
	Other social behaviours	Calf is engaging in social behaviour that is not grooming or social play. This will include head rubbing, head resting, chin resting, suckling, sniffing another calf	**B**
	Feeding/drinking directed behaviour	Includes being fed milk as well as drinking water and eating pelleted food or forage	**F**
	Other secondary behaviour	Calf is engaging in behaviour not noted in the ethogram. This includes interaction with humans - excluding being fed	**X**
	Not visible	Calf is not visible on camera at the time point of analysis and posture or behaviour cannot be determined.	**V**

Table 3: Overall summary statistics (mean and standard error) of untransformed data for each group (data presented as a proportion of time budget [%]). **AP** = assisted placebo, **AT** = assisted treatment, **UP** = unassisted placebo, **UT**= unassisted treatment. Overall proportion of time engaged in lateral recumbency (head position unknown), unknown lying positions, grooming others and other secondary behaviours was less than 0.5% for all groups and data are not presented.

Behaviour	**AP**		**AT**		**UP**		**UT**	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Sternal recumbency head up (NU)	28.5	1.4	31.4	1.3	30.9	1.4	32.9	1.3
Sternal recumbency head down (NH)	49.1	1.5	47.3	1.6	46.5	1.6	47.4	1.3
Sternal recumbency unknown head position (NK)	0.7	0.2	0.2	0.1	0.5	0.2	1.2	0.3
Lateral recumbency head up (RU)	1.8	0.4	1.5	0.3	2.6	0.6	0.7	0.2
Lateral recumbency head down (RH)	5.9	1.0	4.3	0.8	4.7	0.8	2.2	0.6
Attempting to stand (A) - proportion of total time	0.4	0.1	0.4	0.1	0.4	0.1	0.2	0.1
Attempting to stand (A) - proportion of active time	3.2	0.8	3.8	1.0	2.8	1.0	1.6	0.6
Standing (T) - proportion of total time	9.9	0.7	10.5	0.7	10.8	0.7	11.3	0.7
Standing (T) - proportion of active time	72.5	2.8	69.1	2.6	76.7	2.1	76.4	1.7
Walking (W) - proportion of total time	1.7	0.2	2.6	0.3	2.2	0.3	2.0	0.2
Walking (W) - proportion of active time	13.9	1.9	16.1	1.5	14.0	1.7	12.9	1.4
Play (P) - proportion of total time	0.6	0.1	1.2	0.2	1.1	0.2	1.4	0.2
Play (P) - proportion of active time	5.6	1.1	7.0	1.0	6.5	1.1	9.1	1.2
Feeding directed behaviour (F)	0.8	0.1	0.9	0.2	1.1	0.2	1.3	0.2
Grooming self (S)	0.8	0.1	1.2	0.2	0.7	0.1	1.1	0.2
Investigatory behaviours (I)	2.1	0.3	1.9	0.2	1.3	0.2	1.9	0.2
Other social behaviours (B)	0.8	0.2	1.1	0.2	1.0	0.2	1.1	0.2
Total lying in sternal recumbency	78.3	1.4	78.8	1.3	77.9	1.3	81.4	1.1
Total lying in lateral recumbency	7.8	1.2	5.8	1.0	7.2	1.3	2.8	0.7
Total lying in unknown position	1.3	0.5	0.7	0.4	0.5	0.2	0.9	0.4
Total lying	87.4	0.9	85.3	0.9	85.6	0.9	85.1	0.8
Total active behaviours	12.6	0.9	14.7	0.9	14.4	0.9	14.9	0.8
Total secondary behaviours	5.0	0.5	5.6	0.5	4.3	0.4	6.0	0.5
Total head down	55.3	1.5	51.8	1.6	51.3	1.6	49.8	1.3
Total head up	30.4	1.3	33.1	1.3	33.5	1.3	33.7	1.3
Not visible (V)	5.3	1.4	4.8	1.7	2.7	0.8	5.2	2.0

Table 4: Back-transformed mean and standard error and statistical differences (*p*-value and F-statistic) of proportion of time budget (%) engaged in different behaviours for calves born to assisted and unassisted parturition, irrespective of treatment.

Behaviour	**Assisted**			**Unassisted**			**F statistic**	***p*-value**
	Mean (±SE)	**Min (%)**	**Max (%)**	**Mean (±SE)**	**Min (%)**	**Max (%)**		
Attempting to stand (A) – proportion of total time budget	0.230 ± 0.16	0.00	4.17	0.210 ± 0.24	0.00	3.33	0.12	0.728
Walking (W) – proportion of total time budget	2.10 ± 0.06	0.00	8.33	2.20 ± 0.21	0.00	10.0	0.00	0.976
Play (P) - proportion of total time budget	0.80 ± 0.11	0.00	6.94	1.16 ± 0.34	0.00	6.98	**5.61**	**0.019**
Play (P) - proportion of active time budget	5.40 ± 0.12	0.00	50.0	7.14 ± 0.34	0.00	40.0	3.43	0.065
Feeding directed (F)	0.90 ± 0.14	0.00	5.95	1.10 ± 0.17	0.00	8.33	1.45	0.230
Grooming Self (S)	0.90 ± 0.11	0.00	5.95	0.79 ± 0.20	0.00	9.52	0.82	0.367
Investigatory behaviours (I)	2.10 ± 0.05	0.00	11.7	1.50 ± 0.05	0.00	8.33	**4.45**	**0.036**
Total head up	31.3 ± 0.02	5.00	71.9	34.3 ± 0.05	6.25	70.0	**4.33**	**0.038**
Total head down	54.0 ± 0.02	8.77	91.7	49.9 ± 0.05	16.7	85.7	**7.05**	**0.008**
Lying sternal recumbency	78.4 ± 0.01	35.3	100	79.8 ± 0.03	50.0	98.8	1.08	0.299
Lying lateral recumbency	7.60 ± 0.15	0.00	41.2	4.70 ± 0.51	0.00	40.4	3.09	0.080
Total lying behaviours	86.3 ± 0.01	58.3	100	85.4 ± 0.01	57.1	98.8	1.20	0.274
Total active behaviours	13.7 ± 1.04	0.00	41.7	14.7 ± 1.04	1.19	42.9	1.23	0.269
Total secondary behaviours	5.38 ± 0.01	0.00	16.7	4.96 ± 0.02	0.00	19.4	0.58	0.446

Table 5: Back-transformed mean and standard error and statistical differences (*p*-value and F-statistic) of proportion of time budget (%) engaged in different behaviours for calves in each treatment group (ketoprofen/placebo), irrespective of assistance status.

Behaviour	**Ketoprofen**			**Placebo**			**F statistic**	***p*-value**
	Mean (±SE)	**Min (%)**	**Max (%)**	**Mean (±SE)**	**Min (%)**	**Max (%)**		
Attempting to stand (A) – proportion of total time budget	0.223 ± 0.16	0.00	3.39	0.211 ± 0.24	0.00	4.17	1.93	0.166
Walking (W) – proportion of total time budget	2.28 ± 0.12	0.00	10.0	2.03 ± 0.26	0.00	8.93	0.99	0.321
Play (P) - proportion of total time budget	1.18 ± 0.22	0.00	6.94	0.77 ± 0.47	0.00	6.98	**5.72**	**0.017**
Play (P) - proportion of active time budget	7.30 ± 0.23	0.00	40.0	5.20 ± 0.53	0.00	50.0	2.99	0.085
Feeding directed (F)	1.09 ± 0.22	0.00	7.69	0.91 ± 0.25	0.00	8.33	2.38	0.124
Grooming Self (S)	1.04 ± 0.24	0.00	9.52	0.69 ± 0.42	0.00	5.56	**12.45**	**<0.001**
Investigatory behaviours (I)	1.93 ± 0.14	0.00	11.1	1.66 ± 0.26	0.00	11.7	0.28	0.600
Total head up	33.3 ± 0.05	6.25	62.5	32.2 ± 0.12	5.00	71.9	1.00	0.318
Total head down	50.9 ± 0.03	23.3	83.3	53.0 ± 0.04	8.77	91.7	3.14	0.078
Lying sternal recumbency	80.0 ± 0.01	40.0	100	78.3 ± 0.34	35.3	100	1.83	0.177
Lying lateral recumbency	4.46 ± 0.20	0.00	37.5	7.92 ± 0.27	0.00	41.2	**3.79**	**0.052**
Total lying behaviours	85.4 ± 0.01	57.1	100	86.3 ± 0.01	58.3	100	1.41	0.236
Total active behaviours	14.7 ± 0.05	0.00	42.9	13.6 ± 0.11	0.00	41.7	0.99	0.321
Total secondary behaviours	5.70 ± 0.11	0.00	19.4	4.68 ± 0.22	0.00	16.7	**6.56**	**0.011**

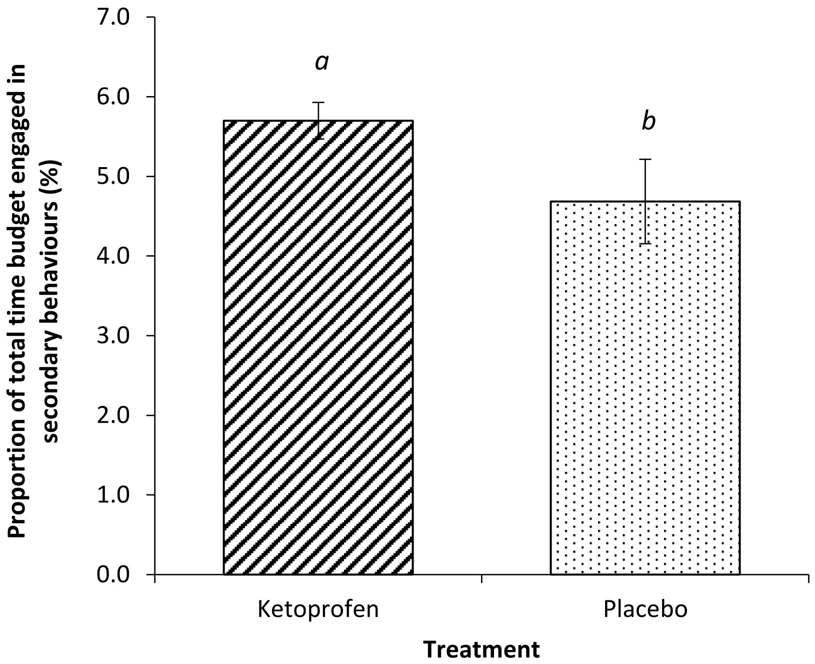

Figure 1: Mean (± SE) percentage of total time budget engaged in combined secondary behaviours for each treatment (ketoprofen/placebo) irrespective of assistance status. Different letters indicate significant differences between treatments (*p*=0.011).

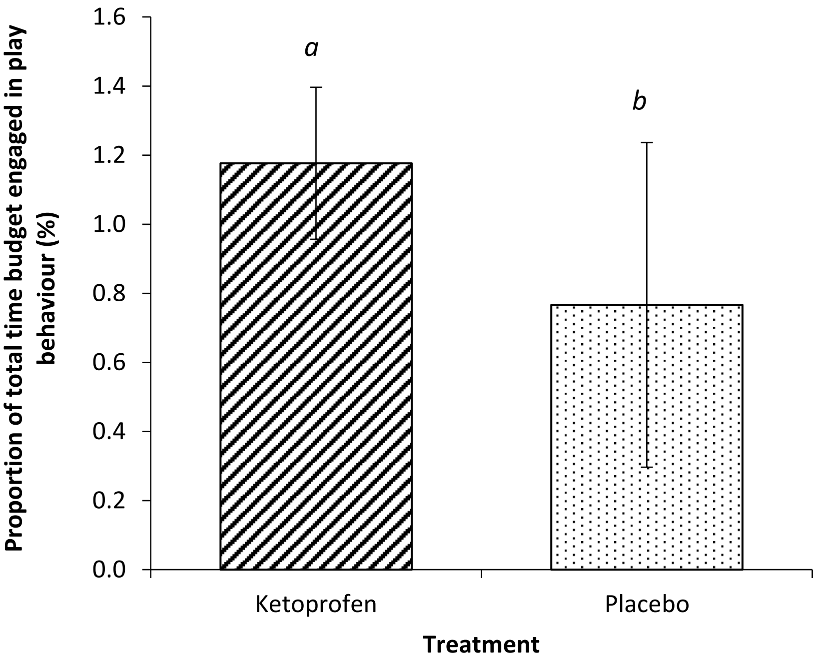

Figure 2: Mean (± SE) percentage of total time budget engaged in play behaviour for each treatment (ketoprofen/placebo) irrespective of assistance status. Different letters indicate significant differences between treatments (*p*=0.017).

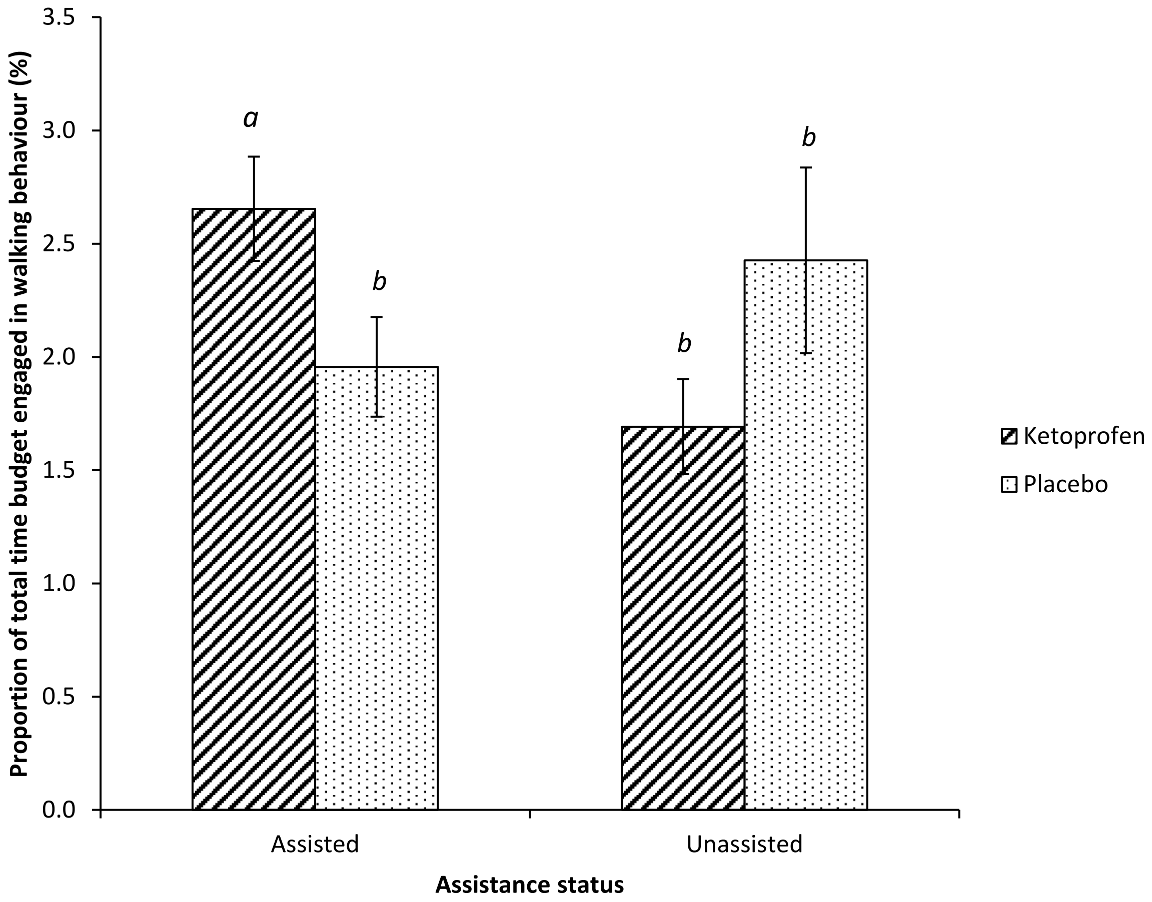

Figure 3: Mean (± SE) percentage of total time budget engaged in walking behaviour for each treatment group (ketoprofen/placebo) by assistance status. Different letters indicate significant differences between groups (*p*=0.004).

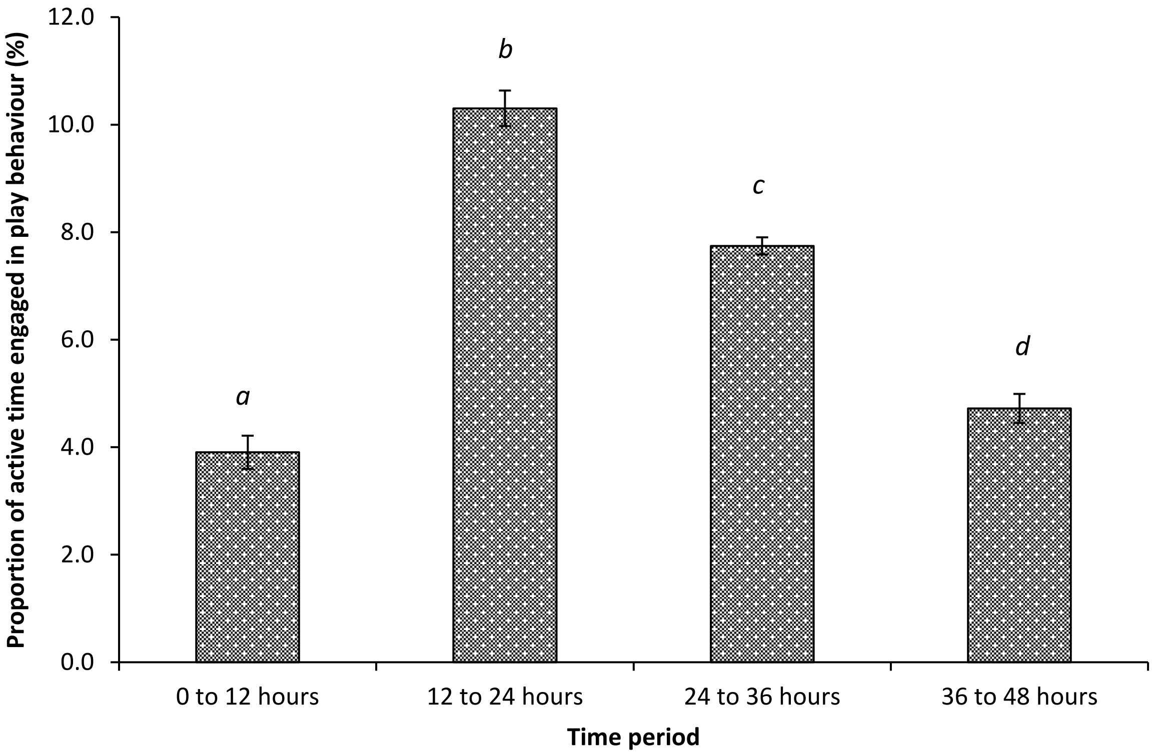

Figure 4: Mean (± SE) percentage of active (non-lying) time budget engaged in play behaviour for each time period, irrespective of treatment or assistance status (*n*=75). Different letters indicate significant differences between time periods (p<0.001).