Weaning management of beef calves with implications for animal health and welfare

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ABSTRACT

Weaning is a necessary husbandry practice in which nutritional, social, physical and psychological stressors are imposed on the beef calf causing alterations in behavioural and physiological responses. The present paper is a review of studies on the influence of weaning stress at housing on beef calves. To date, few studies have examined the effect of weaning on the extended physiological and immunological responses of beef calves and cows. Studies indicate that weaning in combination with immediate housing decreased total leukocyte numbers, reduced in vitro production of interferon-gamma and increased concentrations of acute phase proteins compared with deferring housing for 35 days post weaning. In cows, transitory neutrophilia (increase in neutrophil number) and lymphopenia (decrease in lymphocyte number), reduced interferon-y production, and increased concentrations of acute phase proteins were evident post weaning, whereas post-housing, changes were less marked. Thus, there is a greater transitory reduction in immune function biomarkers in calves immediately post weaning. These immune biomarkers may be used in the future to help identify animals susceptible to weaning stress and that are more likely to succumb to respiratory infection.

Introduction

Under natural conditions, weaning for the offspring involves a complete transition from nutritional and social dependence on the mother to complete independence. This transition is understood to occur gradually as the availability of milk from the mother declines and solid food intake increases, consequently the offspring becomes more socially independent from the mother with greater social interaction with peers (Veissier and Le Neindre 1989; Veissier, Lamy et al. 1990; Veissier, Le Neindre et al. 1990). In farmed animals, the definitive point of natural weaning is somewhat unknown, varying within species and between species and gender (Gonyou and Stooker 1987), and has been reported to be approximately between ten and twelve months of age in Bos indicus cattle (Reinhard and Reinhardt 1981) and Bos taurus, respectively.

In practice, livestock are generally weaned at a much younger age than that observed for natural weaning and, what is more, livestock may also encounter more associated stressors such as social disruption, new environments and additional processing or husbandry practices. In intensive livestock production systems, such as beef production, there is a need to artificially wean calves at approximately six to nine months of age in order to maximize the reproductive potential of the dam as cow-calf separation hastens the return of reproductive cycling and leads to the potential optimal yield of one calf each year. It also allows for the marketing or specialized feeding of the calves (Myers et al. 1999), or in the case of dairy cattle where calves are removed from their dams within hours or days of birth, it allows for an increased amount of milk available for human consumption.

Weaning under modern cattle production systems may be described as a multi-factorial stressor as it incorporates nutritional, physical and psychological elements (Weary et al. 2008). Calves are often subjected to an array of husbandry practices which may compound their stress at the time of weaning. Additional husbandry practices can include; frequent handling and contact with humans, mixing with unfamiliar cattle, movement to new environments whether it be indoor housing or unfamiliar paddocks, switching to different, entirely solid diets, transportation and marketing. Some cattle may also undergo additional processing such as vaccinations, dehorning and castration. Studies suggest that modifying the management of beef calves pre- and post-weaning can reduce weaning stress. Such management practices include the use of anti-suckling devices (nose-clips) for a period prior to weaning (Haley et al. 2005; Boland et al. 2008), fence-line contact between calf and dam post-weaning (Stooker et al. 1997; Price et al. 2003) and a combination of both practices before complete separation (Newberry and Swanson 2008).

Furthermore, research has examined the effect of yard-weaning with and without feed bunk training on the behavioural adaptation of beef calves to a feedlot environment, with particular emphasis on prevalence of BRD (Walker et al. 2007). Calves that were yard-weaned and trained to source grain ration in a trough showed greater feeding behaviour during the first few days in the feedlot compared with those...
that were yard-weaned and not trained, however, this did not result in significantly greater weight gain. Moreover, there was less morbidity in yard-weaned calves compared with pasture-weaned calves following entry into a commercial feedlot (Walker et al. 2007). Compared with beef calves that were weaned in paddocks, yard-weaned calves showed increased social interactions with their peers post-weaning (Fell et al. 1985).

**Neurobiological mechanisms of attachment and separation (mother – young bonding)**

Several neurochemicals are associated with attachment in animals including oxytocin, prolactin, dopamine, gonadal steroids, vasopressin and endogenous opioids. These neuro-transmitters work in synergy to perform specialized functions required for attachment and maintenance of maternal care. Oxytocin is associated with suckling in many mammals and has been shown to be released in calves during nursing but was not released when fed milk from a bucket (Lupoli et al. 2001) indicating that the presence of the dam is required for oxytocin release. Separation-induced calling has been reported to be reduced in oxytocin-deficient mice suggesting a role of this neurotransmitter in attachment-separation behaviour (Winslow and Insel 2000; Insel, 2003) and is a major determinant for bonding of the ewe to her newborn lamb (Kendrick et al. 1997). Working in concert with oxytocin, prolactin promotes maternal care through the activation of dopaminergic and opioid pathways responsible for the addictive and rewarding nature of social contact, lactation and other responses involved in maternal maintenance (Insel et al. 2001; Insel, 2003). Cows’ milk contains α-lactophorin, a tetra-peptide that is structurally similar to endogenous opioids and binds to opioid receptors (Yoshikawa et al. 1986). Some theories suggest that the phases of attachment are similar to that of opiate addiction and withdrawal symptoms (Nelson and Panksepp 1998). The fact that cows’ milk contains opiate-like substances may provide some weight to these theories. Cholecystokinin (CCK) is involved in satiety (Crawley and Crowin 1994), and is increased in calves by non-nutritive suckling following milk consumption (De Passillé et al. 1993). Thus, this peptide may be useful in identifying responses to nutritional and social components of stress.

**Behavioural responses to weaning stress**

Overt behavioural changes, such as increased vocalisations and locomotor activity, caused by weaning, have been documented for several days in the beef and dairy cow and calf, however, these changes are reported to be more intense and persist longer in the calf compared with the cow (Price et al. 2003). A large volume of research has examined these behavioural responses and for the purpose of this paper they are summarized in Table 1. The latency to initiate behavioural responses following weaning varies between species and with age. In general, responses are immediate in piglets (Weary et al. 1999) and foals (McCall et al. 1985), however, this contrasts with longer latency observed in cattle. Additionally, latency of behavioural responses can also be affected by age especially in young calves (Weary and Chua 2000; Flower and Weary 2001). Another point to note on the perturbations to normative behaviours following weaning is that the intensity or magnitude of the responses decline with time. For instance, in abruptly weaned beef calves vocalisations over the first three days after the event were approximately 2000–4000 times greater than those of unweaned control calves (Price et al. 2003). This point was further emphasized by the fact that weaned calves spent 28.1% of their time walking and 23.7% of their time eating compared with 8.6% and 41.1%, respectively, in unweaned control calves. Linked to the intensity or magnitude of the behavioural response is the persistence of such behaviour following weaning. Weaning is unlike many husbandry/management practices in that distress behaviours persist for several days after the event unlike, for example, the relatively short lasting (<5 h) alterations in behaviour observed post-transportation. In the days following weaning, distress behaviours in beef calves lessen (Price et al. 2003; Haley et al. 2005; Boland et al. 2008) and this may signal adaptation by the calves to their new situation. Alternatively, this could also signal an exhaustion of the system to maintain increased locomotor activity and elevated rates of calling, which may be due in part to irritation of the larynx. The greatest changes in behaviour were observed within the first forty-eight (Price et al. 2003; Haley et al. 2005; Boland et al. 2008) and seventy-two hours (Veissier and Le Neindre 1989) after weaning, indicating a peak in the animals’ motivation to reunite.

Studies examining the effects of alternative weaning methods have generally implied that these methods serve to reduce the distress associated with weaning as measured by the reduced behavioural responses compared with abruptly weaned beef calves (Price et al. 2003; Haley et al. 2005; Boland et al. 2008). However, a recent study has questioned the benefits of two-stage weaning with nose-clips and fence-line weaning on calf welfare, concluding that despite the reduction in vocalisations, walking and seeking behaviours observed, calves displayed behaviours synonymous with frustration, resultant from failed suckling attempts distress responses (Enríquez et al. 2010). These authors also showed that there was no overall reduction to distress behaviours for calves weaned by two-stage methods with nose-clips, but rather redistribution over two periods (i.e. in the days post-attachment of nose-clip and days post-weaning).

The differences in behavioural responses in beef calves at abrupt weaning in relation to milk yield availability from their dams have been examined and confirm that dietary intake of milk is a strong component of weaning stress (Ungerfeld et al. 2009). Calves reared on dams producing high milk yields had greater frequency of grazing behaviours post-weaning compared with calves reared on high milk-yielding calves. The extent to which weaning perturbs the behavioural activity in cattle provides evidence that this husbandry practice is stressful. Behaviour does not, however, inform us of the entire physiological consequence of stress experienced by animals (Rutherford et al. 2006; Øverli et al. 2007). Increased behavioural activity that characterizes an animal’s response to weaning is associated with physiological changes that are also indicative of animals under stress (Lefcourt and Elsasser 1995; Broom 2006), and it is these physiological changes
Physiological responses to weaning stress

Endocrine responses

Studies have shown that hormonal indicators of stress are evident in beef calves post-weaning. Following ACTH-challenge, cortisol concentration increased dramatically post-weaning in beef calves and was similar to the increase observed in calves post-transport (Zavy et al. 1992). Crookshank et al. (1979) reported minor increases in concentration of cortisol in beef calves on d 1 post-weaning, which had returned to pre-weaning baseline by d 2 post-weaning. In contrast, Hickey et al. (2003) reported that social group disruption resulted in elevated concentrations of cortisol up to 7 d post-weaning. Concentration of cortisol ranged from 7.5 ng/mL to 16.2 ng/mL at ~168 h pre-weaning and 48 h post-weaning, respectively (Hickey et al. 2003). Additionally, the findings of Blanco et al. (2009) suggest that cortisol remained elevated beyond 7 d post-weaning in beef calves and is not affected by age (abrupt weaning at 90 d of age versus 150 d of age). However, contrary to the findings of Hickey et al. (2003), concentrations did not exceed 8 ng/mL at maximum response Blanco et al. (2009). There was no effect of breed with Pirenaica calves having higher concentrations of cortisol than Parada de Montana calves, post-weaning (Blanco et al., 2009). Breed has been shown to have a significant effect on baseline cortisol levels in beef calves (Zavy et al. 1992). Concentration of cortisol transiently increased from 7.6 ng/mL at the time of calf removal to 11.9 ng/mL at 9 to 12 h post-weaning in beef cows, following which it did not differ from baseline (Whisnant et al. 1985). In terms of catecholamine response to weaning, there is less congruency in the literature on the effects of weaning on these hormones. Findings pertaining to the adrenaline response are equivocal, with increases and no change reported in beef calves post-weaning (Lefcourt and Elsasser 1995; Hickey et al. 2003). Noradrenaline increased immediately (45 min) post-weaning in beef calves (Lefcourt and Elsasser 1995), and was found to be influenced by a weaning × sex interaction, with bull calves having increased concentrations of noradrenaline whereas, it decreased in heifer calves post-weaning (Hickey et al. 2003). With regard to the beef cows, adrenaline increased, whereas noradrenaline was unchanged post-weaning (Lefcourt and Elsasser 1995). Additionally, for other livestock species, weaning has been reported to increase the concentration of cortisol in piglets (Hay et al. 2001; Mason et al. 2003; Kojima et al. 2008), foals (Moons et al. 2005; Fazio et al. 2009) and lambs (Rhind et al. 1998).

Immune cell distribution and function

Extensive studies have demonstrated that stress affects immune cell distribution and functions. Total leukocyte

| Table 1. Changes in behavioural responses following weaning or temporary cow-calf separation in livestock. |
|---|---|---|---|---|
| Animals | Treatment | Results | Reference |
| Salers heifer calves, 7 mo, (n = 30) | 3 treatments: AW-abruptly weaned, CO-controls not weaned, and NS-restricted from suckling, cloth place over dams’ udders. | Weaning resulted in increased social interactions among calves. Increased aggressive events were recorded in calves that were restricted from suckling. Weaned calves chose to remain closest to dam up to 24 d post-weaning, though motivation to suckle had decreased. Compared with female calves, male calves showed weaker preference to their dam compared with another cow. | Veissier and Le Neindre (1989) |
| Salers calves, 8 mo, (n = 67) | Measurement of attraction of calf to dam using choice tests based on time after weaning (0.5, 2, 7, 20 d) and (1, 9, 16, 24, and 35 d). | Treatment differences were greater up to 48 h post-weaning. R vocalized and walked more and spent less time lying than C. | Veissier, Le Neindre et al. (1990) |
| Crossbred beef calves (n = 139) | 2 treatments: C-contact weaned, calves were penned adjacent to dams with which they had visual, olfactory and auditory contact. R-remote weaned-abruptly weaned and penned. | Treatment differences were greatest 20–30 h post-weaning. SP spent more time walking than other treatments. FP and CP spent more time eating than SP and SDNP and FP vocalized less than SP and SDNP. SP spent less time lying than other treatments. | Price et al. (2003) |
| Angus × Hereford calves, mean age (s.d.) 220 (35) d, (n = 100) | 5 treatments: CP-weaned controls at pasture, FP-fenceline contact at pasture following weaning, SP-abruptly weaned at pasture, SDP-abruptly weaned in drylot and preconditioned, SDNP-abruptly weaned, drylot, not preconditioned. | Treatment differences were greatest 48 h post-weaning. Calves weaned using 2-stage weaning vocalized 97% less and spent 79% less time walking, 23% time eating and 24% more time resting than abruptly weaned calves. On d 1, abruptly weaned calves walked 17 km/d, whereas 2-stage weaned calves walked 5.2 km/d. | Haley et al. (2005) |
| Beef calves, mean (s.e) age 184 (13.3) d, (n = 152) | 2 treatments: 2-stage weaning (with nose-clips) vs. abrupt weaning (control) | Treatment differences were greatest 48 h post-separation. Increased vocalisations, walking, butting, and urinating evident in FC and NC compared with CO. Grooming and lying behaviour reduced in FC but not in NC. | Solano et al. (2007) |
| Zebu (B. indicus) male calves, 90 d, (n = 27) | 3 treatments: FC-partially separated from dam with fenceline contact, NC-abruptly separated from dam with no contact, CO-controls remained with dam. Separation lasted 72 h. | After separation, older calves moved more and displayed more explorative behaviours (sniffing walls, bedding, and licking walls) than calves separated at 1d. | Stěhulová et al. (2008) |
| Swedish Red and White and Swedish Holstein calves, age range 20 h – 7 d, (n = 46) | 6 treatments: Separated at 1, 4, or 7 d of age with or without visual and auditory (but not physical) contact of the dam in adjacent pen. | C spent more time idling and walked more and less time eating compared with FL and NC post-weaning. Pre-weaning, NC spent less time eating, more time idling and walked more compared with FL and C. | Boland et al. (2008) |
| Crossbred Angus calves, mean (s.e) age 224 (15) d, (n = 162) | 3 treatments: FL: fenceline contact post-weaning, NC: nose-clip pre-weaning, and C: controls, abruptly weaned | Weaning resulted in increased social interactions among calves. Increased aggressive events were recorded in calves that were restricted from suckling. Weaned calves chose to remain closest to dam up to 24 d post-weaning, though motivation to suckle had decreased. Compared with female calves, male calves showed weaker preference to their dam compared with another cow. | Veissier and Le Neindre (1990) |
number was not altered following abrupt weaning in beef calves that were weaned at 150 d of age, whereas, increased total leukocyte numbers were reported in calves that were weaned at 90 d of age (Blanco et al. 2009). Hickey et al. (2003) reported increased total leukocyte numbers following social disruption of the herd (facilitated by the removal of half the herd; these animals were allocated to abrupt weaning treatment), however post-weaning, there was no difference in total leukocyte number in abruptly weaned and non-weaned (control) calves. In contrast, Phillips et al. (1989) reported decreased total leukocyte number in calves following abrupt weaning. Increased percentage neutrophil and concurrent decreased percentage lymphocytes have been reported in beef calves (Hickey et al. 2003; Blanco et al. 2009) and red deer (Church and Hudson 1999). Weaning age (90 d versus 150 d of age) and breed do not affect the neutrophil:lymphocyte ratio in beef calves (Blanco et al. 2009), however abrupt weaning results in greater increase in neutrophil:lymphocyte ratio compared with gradual weaning in foals (Turner et al. 2003) and wapiti deer (Church and Hudson 1999). The effects of weaning on other cell types such as monocytes, eosinophils, and basophils in beef calves have not been examined. Few studies have examined leukocyte function following weaning. Hickey et al. (2003) reported that in vitro production of IFN-γ was attenuated in weaned beef calves compared with non-weaned calves. A transient reduction in neutrophil phagocytosis has been documented in weaned foals (Sarwar et al. 2008).

**Acute phase protein response**

Findings pertaining to the effects of weaning acute phase protein response in beef calves are not consistent. Although some studies have shown that this response is activated through the measurement of fibrinogen, ceruloplasmin and SAA (Arthington et al. 2003, 2008; Blanco et al. 2009; Carroll et al. 2009), others have reported that concentrations of fibrinogen and haptoglobin are not affected by weaning (Hickey et al. 2003). Further research is needed to identify the most commonly affected acute phase protein in response to weaning stress which would greatly enhance the ability to identify stress susceptible individuals that may require veterinary intervention during periods of stress.

**Metabolites**

Biomarkers of altered nutritional status have been examined during weaning stress in cattle. Altered protein metabolism is evidenced by changes in circulating concentrations of total protein, and its constituents, albumin and globulin, and concentrations of urea. Following weaning, concentration of total protein and urea increased in calves and rapidly returned to pre-weaning concentrations (Phillips et al. 1989). Crookshank et al. (1979) reported no difference in urea concentrations post-weaning. Increased energy metabolism, a hallmark of the stress response as the body diverts energy from processes of a lesser priority to processes required to react to the stressor is evidenced by alterations to concentrations of glucose, NEFA and βHB. Conflicting data exist for the effect of weaning on concentration of glucose, with increases (Phillips et al. 1989) and no differences observed in *Bos taurus* calves post-weaning (Crookshank et al. 1979; Boland et al. 2008). The serum concentration of glucose did not differ post-weaning compared with pre-weaning concentrations in abruptly weaned *Bos indicus* calves (Phillips et al. 1989; Coppo, 2001). Elevated concentrations of NEFA were found on d 1 post-weaning in abruptly weaned, fence-line contact weaned and nose-clip weaned calves, however baseline concentrations were restored by d 7 (Boland et al. 2008).

In Ireland, seasonal grassland-based beef production systems typically comprise a grazing season followed by a period of indoor housing over winter. The majority of calves are spring born and are reared with their dam at pasture and are allowed unlimited nursing, until the end of the grazing season in autumn when they are weaned and generally housed. In non-integrated systems, housing can be preceded by transportation and social regrouping of unfamiliar animals at livestock markets. As such, weaning is an inherent husbandry practice in beef production systems, serving to maximize the reproductive potential of the dam by hastening the return to reproductive cycling and allowing for the marketing and specialized feeding of the calves.

Stress has been linked to many detrimental effects in cattle including immune suppression, increased disease susceptibility and decreased reproductive performance resulting in huge economic losses to the beef industry every year. Weaning has been identified as one of the main stressors implicated in these negative effects due to its multifaceted nature whereby, depending upon the management practices employed, physical, psychological, and nutritional stressors can be imposed on the calf simultaneously. Heightened distress responses and activation of the neuroendocrine-immune axis are hallmarks of stress, and weaning has been demonstrated to induce alterations in behavioural responses (Price et al. 2003; Haley et al. 2005; Enríquez et al. 2010), hormonal mediators of stress (Leffcourt and Elsasser 1995; Hickey et al. 2003) and consequently, alterations in immune function in beef calves (Hickey et al. 2003; Arthington et al. 2005, 2008; Blanco et al. 2009). It is these alterations in immune function that have been associated with increased susceptibility to infection in weaned beef calves, with bovine respiratory disease being the most commonly reported disease (Duff and Galwey 2007). Occurrence of this disease complex has major ramifications in terms of animal health and welfare, and has major economic implications, incurring costs attributable to vaccinations, loss in live weight gains, antibiotic treatments, and mortality (Gunn and Scott 1998; Thomson and White 2006).

**Discussion**

There has been limited research on the effects of weaning alone on beef calves. The majority of the literature has focused on the combined effects of weaning and transportation together since often both of these stressors occur at around the same time in a farm setting. However, abrupt weaning is reported to induce stress in beef calves and is therefore an important area of further investigation (Hickey et al. 2003). Through the examination of these key areas, this paper sought to highlight some of the current gaps in the
knowledge pertaining to weaning stress in beef cattle. Presently, there is somewhat limited information on the physiological, adrenocortical and immunological responses of beef cattle following abrupt weaning. Examination of the effects of weaning on beef cows have been largely neglected by the literature. Research to date has focused on the effects of early weaning, when the calf is approximately 80–90 days of age, and on the nutritional and reproductive status of the cow. Early weaning is implemented in production systems where forage may be limiting due to unfavourable climatic conditions to improve body condition score in beef cows, increasing pregnancy rate and decreasing postpartum an oestrous period (Arthington and Minton 2004).

In a study by Lynch et al. (2010a), a practical consideration regarding post-weaning management practices of beef calves was addressed to ascertain whether a weaning strategy, whereby less stressors are imposed on the calf at the time of weaning could have a positive effect on the physiological and immunological responses post-weaning. Their findings indicated that beef calves that were abruptly weaned and returned to familiar pasture had a less marked stress response than calves that were abruptly weaned and introduced to a new environment of a slatted floor shed and were offered a new diet of grass silage ab libitum plus supplementary concentrates simultaneously (Lynch et al. 2010a). The more marked stress response was attributed to decreased number of lymphocytes, the attenuated production of IFN-γ and greater concentrations of the acute phase protein, fibrinogen, post-weaning in abruptly weaned and housed calves compared with those that were abruptly weaned and returned to pasture. Thus, reducing the cumulative effects of multiple stressors by deferring housing of beef calves for a period of time post-weaning may have beneficial effects on the stressed calf.

In line with the findings of Lynch et al. (2010b) and other studies examining the effects of weaning stress in beef calves (Hickey et al. 2003; Blanco et al. 2009), weaning resulted in neutrophilia and concurrent lymphopenia which was coupled with decreased in vitro production of IFN-γ, and increased acute phase response in beef cows. Thus, it is apparent that the beef cow is stressed by the abrupt separation from her calf. However, when the magnitude and duration of these perturbations in the cow are compared with those in the calf, it appears that stress response is activated to a lesser degree and for a shorter period. The practice of early weaning has not been adopted in Ireland due to favourable climatic conditions for grass growth. Traditionally, cow-calf separation occurs when calves are approximately 7–9 months of age. Observations have confirmed that distinct distress behaviours, such as increased vocalisations and locomotor activity, are expressed by beef cows following abruptly separated from their calves (Price et al. 2003), therefore it is reasonable to assume that physiological and immunological processes similar to those altered in abruptly weaned beef calves would also be altered in beef cows following abrupt separation from their offspring. Additionally, the findings of Lynch et al. (2010a, 2010b) have demonstrated that movement from a pasture environment to a housing environment in a slatted floor shed is capable of inducing a transient stress response in beef calves and cows however, homeostatic mechanisms are capable of regulating this response with no ramifications on animal health or welfare. Collectively, these studies identified a profile in which neutrophilia and concurrent lymphopenia are evident for a brief period following relocation. Due to the pronounced neutrophilia and lymphopenia observed by Lynch et al., (2010a, 2010b), a more in depth examination of the effects of abrupt weaning on neutrophil function and lymphocyte immunophenotypes was undertaken by Lynch et al. (2012). To the author’s knowledge, no other studies have examined these biomarkers in abruptly weaned beef calves. The main findings of Lynch et al. (2012) showed that transient neutrophilia was evident post-weaning, and that this population of circulating neutrophils are less active in terms of phagocytosis than the neutrophil population in circulation prior to weaning (Table 2). Previous studies examining the effects of neutrophils in response to glucocorticoids have reported that neutrophilia is attributed to an influx of immature neutrophils released from the bone marrow into circulation, decreased migration along endothelial cells (Burton and Kehrli, 1996; Burton et al. 2005; Weber et al. 2001; and as evidenced in the work of this review), and by decreased neutrophil apoptosis (Burton et al. 2005; Madsen-Bouterse et al. 2006). The decrease in percentage neutrophils performing phagocytosis post-weaning may be related to the age and stage of maturity of circulating neutrophil at this time. Future work should examine the stage of maturity of neutrophils present at time of neutrophilia in order to determine if these or other factors are responsible for the decrease in bacterial uptake. It is interesting to note that the respiratory burst activity of neutrophils was not affected by weaning, suggesting that these cells are still capable of generating and releasing toxic reactive oxygen species and proteolytic enzymes. Due to decreased apoptosis of neutrophils in response to glucocorticoids, neutrophils have prolonged contact with their localized microenvironment and may secrete noxious agents as the cells proceed through necrosis resulting in localized tissue damage. This paradox, whereby the protective actions of neutrophils may cause tissue damage and exacerbate disease when improperly regulated, has been documented in cattle (Burton et al. 2005) and appears to play a role in overall dysregulated response in stressed cattle (Buckham Sporer, Burton et al. 2007; Buckham Sporer, Xiao et al. 2007). The findings of Lynch et al. (2012) also showed that abrupt weaning induced a redistribution of peripheral lymphocyte immunophenotypes in beef calves. Transient decreases in the proportion of CD4+ , CD8+, and γδ T cells reported immediately post-weaning. Trafficking of lymphocytes is an important and dynamic factor for effective cell-mediated immunity and stress has been shown to influence this process (Kehrli et al. 1999; Viswanathan and Dhabhar 2005; Dhabhar 2009) and has important implications for vaccination protocols (Esser et al. 2003). As stated, these alterations were transient in the beef calf which is in agreement with other studies that reported rapid reversion to pre-stress levels following the cessation of stressor in bovine and rodent studies (Dhabhar et al. 1995; Riondato et al. 2008). Utilising the profile of changes observed in neutrophil number and function and lymphocyte subsets identified by Lynch et al. (2012) as sensitive biomarkers of weaning stress in beef calves, the provision of concentrate supplementation pre-weaning was
examined to determine if this practice could ameliorate the stress response post-weaning.

The findings of Lynch et al. (2012) showed that calves that were offered concentrate supplementation pre-weaning had a lesser degree of neutrophilia compared with calves that were not supplemented pre- and post-weaning. Additionally, a lesser reduction in percentage WC1+ lymphocytes and increased percentage CD4+ lymphocytes were observed post-weaning in supplemented calves compared with calves that were not supplemented. The common alterations in neutrophil number and function, and lymphocytes number and subsets observed are summarized in Table 2. As the predominant lymphocyte phenotype in bovine peripheral blood, CD4+ T cells play an important role directing the immune response towards a cell-mediated, pro-inflammatory or a humoral (anti-body) response via the secretion of specific cytokine subsets by T helper 1 (Th1) or T helper 2 (Th2) cells (Kampen et al. 1999). Th1 cells are involved in cell-mediated immunity whereas Th2 cells are involved in antibody production (Yang and Glaser 2000). A shift away from a Th1 cell mediated response can result in increased susceptibility to viral infections (Elenkov et al. 1996).

The bias for a Th2 response following stress may enhance protection against bacterial pathogens while dampening the response to viral antigens (Salak-Johnson and McGlone 2007). This has important implications for weaned beef calves as BRD is typically characterized by an initial viral infection followed by a secondary bacterial infection. Future work should address the complexity of the relationship between stress and Th1/Th2 balance in weaned beef calves and those likely to succumb to BRD following transportation and housing.

The term biomarker denotes a substance used as an indicator of a biological state and this can be very useful when identifying and defining weaning stress. In terms of potential biomarkers that may be useful to identify stress sensitive beef calves following abrupt weaning, it is evident from the work of this review that no biomarker can be used in isolation, but rather a panel of biomarkers defining a specific profile may be used to identify stressed animals.

Table 2. Summary of the profile of changes in peripheral neutrophil number and function, and lymphocyte number and subsets in beef calves following abrupt weaning.

| Biomarker                  | Lynch et al. (2011)* | Treatment | T × S | Response to weaning | Range | Lynch et al. (2012)* | Treatment | T × S | Response to weaning | Range |
|----------------------------|-----------------------|-----------|-------|---------------------|-------|-----------------------|-----------|-------|---------------------|-------|
| Neutrophils × 10³ cell/µL  | 2 W ***               | ↑↑        | 2.5–6 | Lymphocytes × 10³ cell/µL  | 2 W *** | ↓        | 5.2–7.3 |
| C                         | CS NS                 | ↑         | 2.3–4 | BC                 | NS CS   | ↓         | 5.0–7.3 |
| L-selectin, MFI³           | 2 W ***               | ↓         | 25–40 | CD4⁺, %             | 2 W *** | ↓         | 15–22  |
| C                         | CS NS                 | ↓         | 25–35 | NC                 | NCS     | *        | 21–29  |
| Phagocytosis, %            | 2 W ***               | ↓         | 66–79 | CD8⁺, %            | 2 W *** | ↓         | 8–12   |
| C                         | CS NS                 | ↓         | 66–79 | NC                 | NCS     | ↓        | 8–11   |
| Oxidative burst, %         | 2 W NS                | ↑         | 20–40 | WC1⁺, %             | 2 W *** | ↓         | 12–24  |
| C                         | CS NS                 | ↓         | 23–33 | NC                 | NCS     | ↓        | 10–19  |
| MHC class II⁺, %           | 2 W ***               | ↑↑        | 13–30 | MHC class II⁺, %    | 2 W *** | ↑         | 21–25  |
| C                         | CS NS                 | ↑         | 11    | NC                 | NCS     | ↑        | 11     |

*T × S = Treatment × sampling time interaction.

¹MFI = mean fluorescence intensity. W = abruptly weaned and housed, C = not weaned (controls) and housed, CS = concentrate supplemented pre-weaning, NCS = not concentrate supplemented pre-weaning.
weaning (Lynch et al. 2011, 2012). Hickey et al. (2003) reported that weaning had no effect on concentration of haptoglobin in calves, whereas Arthington et al. (2003) evaluated the effect of weaning and weaning combined with transport in calves and found an increase in the concentration of haptoglobin in calves weaned but not in those weaned and transported, concluding that it is not necessary to have an inflammatory process to increase the concentration of this protein. These authors and the author of this paper acknowledge that husbandry stressors may differentially elicit varied acute phase responses based on the age, breed, and gender of the animal and nature of the stimulus, and thus, further research on the effect of husbandry practices on the acute phase response in cattle is warranted.

Examination of the metabolic profiles, in terms of protein and energy metabolism, in beef cattle post-weaning showed that health and welfare of these animals was not compromised by abrupt weaning. Altered metabolic profiles signal a shift in protein and energy metabolism as the animal adapts to a new diet.

Implications and future work

Reducing the cumulative stressors at the time of weaning may allow for smoother transition from social and nutrition dependence on the dam to complete social and nutritional independence of the calf post-weaning. Manipulation of pre- and post-weaning management practices can reduce weaning stress as evidenced by the deferral of housing of a period post-weaning and by the provision of concentrate supplementation pre-weaning. The practicality of these strategies will be dictated by environmental and economic factors, namely grass availability at pasture and the costs associated with feeding supplementary concentrates pre-weaning.

It is evident from the findings of Lynch et al. (2011, 2012) that homeostasis is altered at numerous levels which may affect immunocompetence in beef calves. Extended and more profound perturbations to neutrophil number and function, greater than those observed in this review, may induce a state, whereby the protective actions of these phagocytosing cells begin to damage tissue, thus, allowing opportunistic bacteria to become resident locally. Moreover, adaptive immune responses may be attenuated by the redistribution of lymphocyte subsets reducing the capacity for surveillance of foreign antigens. Both these factors could influence the efficacy of vaccination protocols in weaned beef calves.

Due to changes observed in peripheral immune responses post-weaning in calves, it would be worthwhile to investigate whether these changes reduce immunocompetence when calves are challenged with pathogens. These studies could incorporate in vivo challenge models where calves are challenged with viral strains associated with BRD, such as BRSV or PI-3 or IBR with and without subsequent challenge with bacterial agents, such as M. haemolytica. In order to determine immunocompetence, viral titres and specific antibodies against the viral strain employed in challenge model could be measured.

A combined approach using genomic, cellular and proteomic analyses could investigate the effects of weaning on candidate genes that have been reported to be altered by other husbandry practices in cattle (e.g. transportation). These studies could focus on genes relating to innate immune response, apoptosis, signal transduction, and wound healing. Future work investigating in vitro leukocyte function and cytokine and chemokine profile characterization in weaned beef calves could provide vital information on the key mediators in the stress-immune axis and how this response is regulated in cattle.

To gain a greater understanding of the complex interactions between stress and the immune system in cattle, in vitro and ex vivo studies could be carried out to examine the pro-inflammatory cytokine signalling directing the response. Cytokine responses could be measured using a candidate gene approach and quantitative real-time PCR and by intracellular staining techniques utilizing flow cytometry.

In light of the synergistic actions of viral and bacterial pathogens in which cattle become infected with BRD, it would be of interest to address the complexity of the relationship between weaning stress and the Th1/Th2 balance. In the future, it may be possible to identify a greater number of significant biomarkers and to use both in vivo and ex vivo models to gain a greater understanding of stress related disease susceptibility in weaned beef calves.

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Availability of data and materials

All data are included in the article.

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