END OF PROJECT REPORT

The effect of abrupt weaning of suckler calves on the plasma concentrations of cortisol, catecholamines, leukocyte, acute-phase proteins and *in vitro* interferon-gamma production.

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Grange Beef Research Centre

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1. Summary

The objective of this study was to examine the effect of abrupt weaning (inclusive of social group disruption and maternal separation) on the physiological mediators of stress and measures of immune function. Thirty-eight male and 38 female continental calves were habituated to handling for two weeks prior to bleeding. Calves were blocked on sex, weight and breed of dam and randomly assigned, within block, to either a control (cows remain with calves) or abruptly weaned group (calves removed from cows). Animals were separated into the respective treatment groups at weaning (0 h). Calves were bled at – 168 h, 6 h (males only), 24 h, 48 h and 168 h post weaning. At each sampling time an observer scored the behavioural reaction of calves to sampling. Blood samples were analysed for cortisol, catecholamine concentrations (not sampled at –168 h) and *in vitro* interferon-gamma production, neutrophil :lymphocyte ratio and acute phase protein concentrations. All continuous data were analysed using a split-plot ANOVA, except that collected at 6 h, which was analysed using a single factor ANOVA model. The effects of weaning, calf sex and time and respective interactions were described. Disruption of the established social groups at 0 h, increased ($p<0.001$) the plasma cortisol concentration and neutrophil: lymphocyte ratio and reduced the leukocyte concentration ($p<0.001$) and the *in vitro* interferon-gamma response to the mitogen concanavalin-A ($p<0.001$) and keyhole limpet haemocyanin ($p<0.001$) for weaned and control animals, when compared with –168h. Plasma adrenaline and noradrenaline concentrations were not affected by group disruption. There was no effect of weaning or sex on calf behavioural reaction to handling during blood sampling. Plasma cortisol and adrenaline concentrations were not affected by weaning or sex. Plasma noradrenaline concentration was influenced by weaning x sex ($p<0.05$) and time x sex ($p<0.05$). The response increased for male calves with weaning and increased with each sampling time post weaning. For heifers the response was not affected by weaning and plasma concentrations decreased at 168 h post weaning. There was no effect of weaning or sex on leukocyte concentration. The neutrophils : lymphocyte ration increased post weaning ($p<0.01$) and was affected by sex ($p<0.05$). Weaning decreased ($p<0.05$) the *in vitro* interferon-gamma response to the antigen KLH. There was a time x weaning x sex ($p<0.05$) interaction for fibrinogen concentration but no effect of treatment on haptoglobin concentration. Abrupt weaning increased plasma cortisol and nor-adrenaline concentrations, which was accompanied by

attenuation of *in vitro* interferon gamma production to novel mitogen and antigen complexes up to 7 days post weaning.

Key words

Calves, catecholamines, cortisol, immunity, neutrophil :lymphocyte ratio, weaning stress.

2. Introduction

Previous studies have examined the effect of maternal separation under varying management regimens on calf behaviour (Veissier et al., 1990a), plasma acute phase protein concentrations (Phillips et al., 1989) and neutrophil: lymphocyte (N: L) ratio (Church and Hudson, 1999). These results indicate that breaking the maternal bond is stressful to the calf. Stressful events have previously been associated with attenuation of immune function (Blecha et al., 1984; Sevi et al., 2001) and disease susceptibility in animals (Roth, 1982; Burton et al., 1995). MacKenzie et al. (1997) found no effect of abrupt weaning on the humoral immunity of weaned calves. Attenuation of the cell mediated (Hickey et al., 2003) but not humoral immunity (Fisher et al., 1997a) has been identified in situations of chronic stress. Pollock et al. (1991) also suggested that the cell mediated, rather than humoral immunity, may be a more reliable indicator of the physiological status of calves older than 5 months. The adrenal hormones are recognised indicators of stress in bovine models (Toates, 1995) and no work has been identified which describes the long-term effect of weaning on the mediators of stress. The objective of this study was to examine the effect of abrupt weaning (inclusive of social group disruption and maternal separation) on the physiological mediators of stress (cortisol, adrenaline and noradrenaline) and measures of immune function (*in vitro* interferon-gamma production, neutrophil :lymphocyte ratio and acute phase protein concentrations). The hypothesis of this study was that the abrupt weaning of calves is a stressor, which will increase the physiological measures of stress hormones, resulting in attenuation of the cell mediated immunity.

3. Materials and Methods

Animal Management

At the start of the grazing season, suckler cows were blocked on breed of dam (Limousin x Friesian, 0.75 Limousin, Limousin x Simmental, Limousin, Charolais) and parity and assigned within block to one of four herds. There were two herds of male

calves (mean liveweight 253 ± 43 kg) and two herds of female calves |(mean liveweight 239 ± 49 kg). Calves single suckled their dams over the 7-month grazing season on a perennial ryegrass sward. One week prior to weaning all calves were weighed. Within each herd, 36 of the calves were blocked on weight and breed of dam and assigned within block, to either a control © or abruptly weaned (W) treatment group. For each herd on the morning of weaning, the dams of calves assigned to W were moved to slatted accommodation and the calves (n=18) placed alone in a grazing paddock. The housing facility was a sufficient distance removed such that the vocalisations of either group could not be heard by the other. For the control group, both dams (n=18) and calves (n =18) were placed in a grazing paddock which was a sufficient distance removed from the animals of W such that their vocalisations could not be heard. All grassland areas were of similar herbage quality and quantity. The experimental duration extended to 7 days post weaning.

Animal handling

Two weeks prior to the start of the study, calves were habituated to handling. During this time on alternate days, each calf was placed in a crush adjacent to the area of grazing for a total duration of 15 minutes. Within this time allocation, the calf was haltered, restrained and acclimatised to human interaction on 3 occasions.

Behavioural score

At each blood sampling time (described below) an observer scored the reaction of calves to handling at three distinct stages, while in the crush. Stages were separated by a 2 minute time lapse. These stages were identified as the animals reaction to restraint by a halter, the animals reaction to jugular clamping and the animals reaction to jugular venipuncture. An animal was given a score of 1 if it reacted to the presence and actions of the human at any stage but calmed once the individual moved away or in the case of stage three, once the needle was inserted. An animal scored 2 if it continued to react at any one of the identified stages but subsequently calmed during the intervening 2 min. before the procedure moved to the next stage. An animal scored 3 if it continued to react at any two of the identified stages but subsequently calmed during the intervening 2 min. before the procedure moved to the third stage. An animal scored 4 if it continued to react during all stages. The intensity of the reaction for stages 2 – 4 was also scored. The reaction scored 1 (mild) if the animal stepped in any direction but never pulled against the restraining rope. The reaction scored 2 (intense) if the animal pulled against the restraining rope whilst moving or stationary. The reaction scored 3 (severe) if the animal attempted to turn while restrained or went down on fore and/or hind legs. The final score attributed to each animal at each bleed was calculated as score of stage x score of reaction (for stage one the score was automatically 1).

Physiological measurements

All calves were blood sampled by jugular venipuncture at –168 h, 24 h, 48 h and 168 h post weaning. Due to management constraints (lack of daylight at outfield bleeding pens) only the bull calves were blood sampled at 6 h post weaning. Samples were collected into lithium heparinised tubes and plasma harvested after centrifugation at 3000 g for 10 min. and frozen. Plasma was subsequently analysed for cortisol concentration using a radioimmunoassay (Corti-Cote, ICN Pharmaceuticals, NY, USA). This assay was adapted and validated for bovine plasma (Fisher et al., 1996), with an intraassay CV for samples containing 5.5, 18.7 and 70.7 ng of cortisol/ml of 13.3, 10.1 and 9.1%, respectively and an interassay CV for the same samples of 17.5, 11.3 and 10.8%. For catecholamine analysis, 5 animals were randomly selected from each treatment group, within each herd. These calves were blood sampled by jugular venipuncture at 6 h (males only), 24 h, 48 h and 168 h post weaning, into heparinised tubes. Samples were

immediately transferred to heparinised tubes containing 5 mg sodium metabisulfite and transferred to the laboratory on ice. Plasma was harvested within 1 h of collection and frozen at –70 C. Catecholamine were quantified using a HPLC assay based on the method of Lefcourt and Elasser (1995).

Immune measures

Blood samples were collected at –168h h, 6 h (males only), 24 h, 48 h and 168 h post weaning. Blood samples collected into lithium heperanised tubes were centrifuged at 3000 g for 10 min. and the plasma frozen. Plasma was analysed for haptoglobin levels (validated for bovine plasma by Skinner et al., 1991) using an appropriate assay kit (TP801, supplied by Tridelta Development Ltd., 48 Main St., Bray Co. Wicklow). Blood samples collected into sodium citrate tubes were centrifugated at 3000 g for 10 minutes and the plasma frozen. Plasma fibrinogen was measured using an appropriate assay kit (524484, Roche-Boerhinger, Mannheim, Germany). Blood samples were collected into a K_3-EDTA tubes and the leukocyte profile (total white blood cells concentration and lymphocyte and neutrophil proportions) for each animal was described using the May-Grunwals/Giemsa stain of eosine-methylene blue, modified for microscope and conducted by the National Equine Centre, Ireland. The stimulated lymphocyte production of interferon-gamma was determined following whole blood culture of heparinised plasma in the presence of keyhole limpet haemocyanin (KLH) antigen and Concanavalin A (Con-A) mitogen (Wood et al., 1990). The interferon-gamma production responses, were quantified *in vitro* using an ELISA procedure (Rothel et al., 1990; CSL Biosciences, Parksville, Victoria, Australia). In brief this procedure required that duplicate 1.48-ml aliquots of blood were cultured in 24-well culture plates (Costar Corporation, Cambridge, MA) with 20 μl of PBS containing either 1mg/ml of KLH or 1.0 mg/ml of Con-A or no additive, for 16 h at 37 ^{o}C and in an atmosphere of 5 % CO_2 in air. The culture plates were then centrifuged and the supernatant harvested and frozen at –20 ^{o}C until it was assayed for interferon-gamma production using an ELISA procedure (BOVIGAM, Biocor Animal Health). The *in vitro* KLH- and Con-A-stimulated interferon-gamma production was calculated by subtracting the absorbancy at 450 nm of wells that received PBS alone from the absorbancy of wells that received either KLH or Con-A, respectively.

Statistical analysis

The data for all blood measures, except those sampled at 6 h, were analysed in nine randomized blocks by a split-plot ANOVA. The main plot included effects of weaning, sex and weaning x sex. The sub-plot included the effects of time, sex x time, weaning x time and sex x weaning x time. A diurnal variation exists in plasma cortisol and leukocyte profiles. Therefore data on all blood measures pertaining to the 6 h sampling time were analysed separately in nine randomized blocks by a single factor ANOVA, which described the effect of weaning. Following a significant F-test, Fishers least significant difference test was applied to, determine statistical differences between treatments (Snedecor and Cochran, 1989). Data on temperament score were analysed using a Friedman two-way analysis to describe the effect of sex and weaning (Steel and Torrie, 1960). Linear and quadratic regressions were fitted to determine the relationship, if any, between behavioural score and plasma adrenaline, noradrenaline and cortisol concentrations.

4. Results

There was no effect of sampling time, weaning or sex on the measured behavioural score of calves (Table 1.) There was no effect of time, weaning or sex on plasma adrenaline concentration (Table 2). There was no effect of weaning on the plasma adrenaline response of bull calves at 6 h post-weaning (Table 3). Plasma cortisol concentration increased after social group disruption at weaning ($p<0.001$, Table 2), but not by weaning or calf sex or by weaning at 6 h (Table 3). Plasma noradrenaline concentration was influenced by weaning x sex ($p<0.05$, Table 2) and time x sex ($p<0.05$) interaction, as the noradrenaline response of bulls only increased with weaning and time. This increase was evident ($p<0.01$, Table 3) at 6 h. The response for heifers was influenced by a sharp decrease in plasma concentration at 168 h post weaning, which was evident in both the control and weaned animals.

There was a linear ($p<0.05$) and quadratic ($p<0.05$) relationship between behavioural score and cortisol concentration, which accounted for 2.3 ands 2.5 % of variance, respectively. There was a linear ($p<0.05$) and quadratic ($p<0.05$) relationship between behavioural score and noradrenaline concentration, which accounted for 3.3 and 4.0 % variance, respectively. There was a linear ($p<0.01$) and quadratic ($p<0.01$)

relationship between behavioural score and adrenaline concentration, which accounted for 8.2 ands 7.4 % variance respectively.

There was no effect of weaning or sex on total white blood cell concentrations (Table 4). Social group disruption increased ($p<0.001$) the total leukocyte concentration when compared to 24 h and 48 h, but not at 168 h post weaning. In respect of lymphocyte proportion, male calves had a lower ($p<0.05$) proportion than female calves, and weaning decreased the lymphocyte proportion at 24 h, 48 h and 168 h post weaning ($p<0.05$). This effect was also evident ($p<0.01$, Table 3) at 6 h. The neutrophil proportion was higher ($p<0.05$) for male than female calves. The time x weaning ($p<0.01$) interaction described an increase in the neutrophil proportion for weaned animals at 24 h and 168 h post weaning. This increase was also evident for bull calves ($p<0.01$) at 6 h. The neutrophil:lymphocyte (N:L) ratio was higher ($p<0.05$) for male than female calves. Weaning increased ($p<0.01$) the N: L ratio. The N: L ratio was higher at 24 h but not 48 h or 168 h post weaning when compared with the pre-weaning value. Weaning increased ($p<0.05$) the N: L ratio for bull calves at 6 h post weaning.

The *in vitro* interferon-gamma response to the mitogen Con-A was reduced ($p<0.001$) after social group disruption (Table 5) and did not recover by 168 h post weaning. The response to the mitogen KLH was also reduced ($p<0.001$; Table 5) after social group disruption and did not recover after 168 h post weaning. The KLH response for weaned calves was lower ($p<0.01$) than that measured for control animals, and was lower at 6 h ($p<0.05$) for weaned bull calves when compared with controls.

There was no effect of sampling time, weaning, or sex, on calf plasma haptoglobin concentration. There was a time x weaning x sex interaction ($p<0.05$) for plasma fibrinogen concentration which is described in Figure 1. For the unweaned males, the plasma fibrinogen concentration was highest ($p<0.05$) at –168 h. The fibrinogen concentration was elevated ($p<0.05$) at 48 h when compared with 24 h, but concentrations declined at 168 h ($p<0.05$) such that they did not differ from values obtained at 24 h. For weaned males, there was no effect of social disruption on the fibrinogen levels. However plasma concentrations increased at 48 h ($p<0.05$) when compared with levels recorded at 24 h and subsequently decreased at 168 h ($p<0.05$). Plasma concentrations of fibrinogen were higher for male weaned calves than control animals at 24 h, higher than all treatment groups at 48 h and higher than the female control group at 168 h post weaning. For female controls and weaned calves the plasma fibrinogen concentration decreased at 24 h

($p<0.05$) post weaning, increased at 48 h ($p<0.05$) and decreased at 168 h post weaning ($p<0.05$).

5. Discussion

Management stress is ypothalam as one of the causative factors of many clinical conditions in bovines (Roth, 1982; Burton et al., 1995, Burton and Kehrli, 1995), with important economic implications (Gunn and Scott, 1998, Lekeux, 1995). Two main stress pathways, whose response functions are central to survival, are also central to the stress-immune axis activation that can lead to disease susceptibility (Riad et al., 2002, Eskandari and Sternberg, 2002). The first, activation of the hypothalamus-pituitary-adrenal axis (HPA axis), results in a significant release of the steroid hormone, cortisol, from the cortex of the adrenal glands. Systemic cortisol concentrations increase several minutes after perceived threat and can last for a number of hours. Cortisol is a potent glucocorticoid (Riad et al., 2002), the immunosuppressive effects of which may serve as physiological down-regulators of initiated immune responses during recovery from infection or tissue damage (O'Connor et al., 2000). The second major pathway mediating physiological stress responses in animals is activation of the sympatho-adrenal axis (Minton, 1994; Morberg, 1997; O'Connor et al., 2000). This results in release of the adrenergic neurotransmitters, adrenaline and noradrenaline, from the sympathetic nerves and medullae of the adrenal glands. Sympatho-adrenal axis activation occurs in seconds relative to perceived threats (Erikson et al., 1999). All leukocytes express the β-adrenergic receptors that bind adrenaline and noradrenaline (Abo and Kawamura, 2002, Nagatomi et al., 2000, Black, 1994). However, a paucity of information exists relating to the physiological roles of these neurotransmitters in immune function. This is attributed to the extreme difficulty in sampling and measuring blood ypothalamic s, caused by their sensitivity to animal handling during sampling and short half life (Minton, 1994). Past studies, have documented clear changes in circulating numbers of neutrophils and lymphocytes, attenuated proliferation of T and B lymphocytes, and modified expression of surface adhesion and antigen presenting molecules on leukocytes subjected to stress hormones *in vivo* and *in vitro* (Murata et a*l*., 1985, Burton et al., 1995, Burton and Kehrli, 1995, Anderson et al., 1999, Earley and Crowe, 2002, Hickey et al., 2003). The hypothesis of this study was that the abrupt weaning of calves is a management stressor,

which will increase the physiological measures of stress hormones, resulting in attenuation of immune function.

Unfamiliar human-animal interaction can create a feeling of unease, which is associated with a sympathetic adrenomedullary and/or the ypothalamic response by the animal (Mason, 2000). Therefore calves were habituated to both the restraining and sampling environment, and handling such that the hormonal response to handling would be minimised, as would be the resulting impact on measures of immune function. This approach is supported by the work of Lensink et al. (2000) and Bovin et al. (2000) who indicated that animals have the ability to adapt to human interaction. The behavioural response of each animal during the sampling procedure was evaluated at 3 distinct periods (restraint, clamping and venipuncture). The animal behavioural score did not change with time, which suggests that calves had habituated to the environment and handlers prior to the start of the study. Also the lack of any sex effects in the light of findings from Lanier *et al*. (2000) who reported that heifers were found to be more excitable than steers in an open mart environment, would support habituation. Though there were significant linear and quadratic associations between behavior and stress hormones; the low experimental variation accounted for in each of these relationships was not biological relevant. This would suggest that the physiological and immunological responses measured in this study were dominated by the imposed treatments rather than animal handling.

Bonding behaviour between dam and calf, and between calves within social groups has been established through behavioural observations. The stability of maternal (Veissier et al., 1990b) and social counterpart (Veissier and Le Neindre, 1989) relationships are important for young calves. Abrupt weaning not only disrupts the maternal bond between the calf and its dam, but also the social bond between the animal and their familiar social group. In this study the disruption of the established social group, an inherent aspect of weaning, increased the plasma cortisol levels for all calves up to 168h post weaning. Though transient diurnal alterations in cortisol concentration occur, more sustained increases are associated with situations of stress. The authors are not aware of any study reporting cortisol levels over a 7-day duration, post psychological stress. Minton and Blecha (1990) reported that though plasma cortisol levels increased within 15 min of the restraint and isolation of sheep for 6 h (up to 40 ng/ml), levels remained elevated (18 ng/ml) when compared with controls (< 10 ng/ml) for up to 24 h post treatment, while Minton et al. (1992) reported that where a psychological stress was repeated over 3 days, an attenuation of the cortisol response occurred. In the current study

levels remained elevated for up to 168 h post weaning when compared with –168 h, though noticeably declining. Plasma cortisol concentrations were not affected by weaning or calf sex in this study, which was supported by Lefcourt and Elasser (1995). Due to the periodic sampling procedures used in the current study, to facilitate minimal animal handling, it cannot be ascertained if there was also a peak in plasma cortisol concentration. In other models peak cortisol levels were recorded at 15 min post regrouping and relocation (Sevi et al., 2001) or branding (Lay et al., 1992) and 43 min post castration (Earley and Crowe, 2002).

Baseline levels of noradrenaline are consistently higher than that of adrenaline in bovine studies and a complementary increase in adrenaline and noradrenaline was reported by Lefcourt and Elasser (1995). These authors reported that the temporary separation of a 4-6 month old calf from its mother increased both the adrenaline (max. 0.13 ng/ml) and noradrenaline (max. 0.08 ng/ml) response when separated for 24 h but not for 45 min., with a subsequent decrease on re-unification. The experimental procedure involved the temporary restraint of the calf in isolation followed by physical but not audio-visual separation for 24 h. In the present study abrupt weaning did not elevate the plasma adrenaline concentration of calves, but did increase the noradrenaline response when compared with control animals. Discrepancies between the findings of Lefcourt and Elasser (1995) and the present study, may therefore be attributed to differences in age at separation, breed or particularly animal management, as the animals in this study were subjected to continued rather than intermittent separation from the dam. Koob (1999) suggests an associative interaction between cortisol releasing factor and noradrenaline release in the locus coeruleus of the brain in response to stress, where their interaction is important in mediating behavioural responses to the stressor. In response to this activation noradrenaline can be released to the peripheral pool from storage vesicles in the sympathetic nerves system, where its concentration is estimated to be 90% greater than that of adrenaline (Flatmark, 2000). In light of this, the findings of this study suggests that weaned bull calves experienced a greater difficulty in dealing with abrupt weaning than females, as noradrenaline homeostasis had not re-established at the last sampling time, which occurred 7 days post weaning.

Past studies have examined phenotypic alterations of blood leukocytes as potential biological indicators of physiological stress and disease susceptibility in animals. Anderson et al. (1999) reported an increase the N: L ratio when animals were challenged with dexamethasone. In this study the glucocorticoid rather than the catecholamine

response may be the contributing factor to the alteration of the N: L ratio, as noradrenaline levels remained high for the bulls, while the N: L ratio returned to levels similar to –168 h by 48 h. Church and Hudson (1999) reported an increase in the N:L ratio up to 14 days post dam removal for calves of the Cervus elaphus species. These calves were not habituated to handling and may have reacted to the collection and sampling procedures at each sampling interval (d1, d7 and d14), with attenuation of the response occurring at the later period (d28). This in turn may have extended the measured increase in the N:L ratio. In this study fluctuations in the N: L ratio did not affect the Con-A response and were not solely responsible for attenuation of the KLH response which remained depressed despite the normalisation of N: L by 48 h.

The production of interferon-gamma is associated with subsets within the CD4 T lymphocyte family (Wood and Seow, 1996), where the production of the cytokine is stimulated by mitogen/antigen challenge. The KLH antigen is a non-specific immune response modifier, which can induce both a cell-mediated and a humoral response (Harris and Markl, 1999), while Con-A is a specific T cell mitogen. The interferon-gamma response for antigen and mitogen challenge was attenuated by social group disruption , and by weaning for the KLH response only. Though the cortisol concentrations recorded in the present study were within the diurnal variations in peripheral cortisol reported by Lefcourt et al. (1993, 1 – 17 ng/ml), the increase from 7.4 to 14.2 ng/ml recorded during group disruption was associated with this decrease in cell mediated immune function. The continued elevation of the cortisol response was also associated with the continued attenuation of both the Con-A and KLH response. It is possible that an alteration in the lymphocyte concentration and conceivably sub-population profiles, was associated with the glucocorticoid response at group disruption (Anderson et al., 1999), though the influence of glucocorticoids on cell mediated immunity *per se* has been questioned (Fisher et al., 1997b; Anderson et al., 1999). It has been proposed that catecholamine production can influence immune function both at the tissue and cellular level through innervation and receptors respectively (Minton, 1994; Qui et al., 1996; Ansted-Michael, 1998). Both noradrenaline plasma concentration and interferon-gamma production in response to KLH were influenced by weaning. However the lack of a significant sex effect on the KLH response would question if the continued increase in noradrenaline levels for bulls may be associated with this depression. As KLH is a non-specific antigen its peripheral effects may therefore be associated with B cell function. However previous

studies have shown no effect of chronic stress (Fisher et al., 1997a) or weaning stress (MacKenzie et al., 1997) on humoral immunity post-KLH challenge.

Bacterial infections and physical traumata can lead to a drastic change in the synthesis of acute-phase proteins in the liver. Monitoring the circulating concentration of acute phase proteins is important in animal health evaluation, though it is recognized that there are substantial differences between species in the physiological acute phase protein response following stimulation (Conner et al. 1988b, Conner et al. 1988a, Eckersall et al. 1996). Haptoglobin is a major acute phase protein in cattle where plasma concentrations can change from negligible circulating levels in healthy animals, to increases of 100 fold on stimulation/infection (Wittum et al., 1996, Fisher et al., 1997b, Earley and Crowe, 2002). This measure, along with fibrinogen was therefore used in this study to determine the health of each calf during the post weaning observation period. The effect of weaning was associated with alterations in plasma fibrinogen but not haptoglobin concentration. Weaned bulls had higher plasma fibrinogen concentration when compared with all other groups, but like other groups the plasma concentrations had returned to, or were less than pre-weaning values by 168 h. The measured range of fibrinogen was higher than that measured in animals dealing with long-term physical stress (Hickey et al., 2003) but did not differ greatly from ranges reported by Earley and Crowe (2003) in non-stressed Friesian calves. The animals therefore should no sub-clinical signs of poor health up to 7 days post weaning, in an outdoor environment.

In this study physiological alterations in cortisol and noradrenaline concentration and attenuation of immune function indicated that abruptly weaned suckler calves are sensitive to the social stress associated with group disruption and weaning. It may be concluded from this study that the *in vitro* Con-A response was sensitive to social disruption and mirrored that of cortisol, while the *in vitro* KLH response was sensitive to weaning stress which also influenced the peripheral noradrenaline concentration.

6. Implications

As animals in the present study were habituated to handling the reaction of ‘on-farm’ suckler calves to weaning may be greater. As the alterations in immune function and hormonal mediators of stress were still evident 7 days post weaning, farm management practices at weaning should aim to minimise the social distress of calves during this time and allow calves a period of adaptation before other management stresses are imposed.

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7. References

Anderson, B. B., D. L. Watson, and I. G. Colditz. 1999. The Effect of Dexamethasone on Some Immunological Parameters in Cattle. Veter. Commun. 23:399–413.

Ansted-Michael, I., A. Hunt, L. Carlson, and K. Burli. 1998. Variability of peripheral blood lymphocyte beta-2-adrenergic receptor density in humans. Amer. J.. Respir. Crit. Care Med. 157:990-992.

Blecha, F., S. L. Boyles, and J. G. Riley. 1984. Shipping Suppresses Lymphocyte Blastogenic Responses in Angus and Braham x Angus feeder calves. J. Anim. Sci. 59:576-583.

Boivin, X., H. Tournadre, and P. Le Neindre. 2000. Hand-feeding and gentling influence early-weaned lambs' attachment responses to their stockperson. J. Anim. Sci. 78:642-652.

Burton, J. L., and M. E. Kehrli, Jr. 1995. Regulation of neutrophil adhesion molecules, and shedding of Staphylococcus aureus in milk of cortisol- and dexamethasone-treated cows. Am. J. Vet. Res. 56:997-1006.

Burton, J. L., M. E. Kehrli, Jr., S. Kapil, and R. L. Horst. 1995. Regulation of L-selectin and CD18 on bovine neutrophils by glucocorticoids: effects of cortisol and dexamethasone. J. Leuk. Biol. 57:317-325.

Church, J. S., and R. J. Hudson.1999. Comparison of the stress of abrupt and interval weaning of farmed wapiti calves (Cervus elaphus). Small Rum. Res. 32:119–124.

Conner, J.G., P.D. Eckersall, J. Ferguson and T. A. Douglas. 1988a. The acute phase response in the dog following surgical trauma. Res. Vet. Sci. 45:107-110.

Conner, J.G., P. D. Eckersall, A. Wiseman, J. Ferguson, and T. A. Douglas. 1988b. Bovine acute phase response following turpentine injection. Res. Vet. Sci. 44:82-88.

Earley, B., and M. A. Crowe. 2002. Effects of ketoprofen alone or in combination with local anesthesia during castration of bull calves on plasma haptoglobin, *in vitro* interferon-γ production, white blood cell numbers and animal performance. J. Anim. Sci. 80:1044–1052.

Eckersall, P. D., P. K. Saini and C. McComb. 1996. The acute-phase response of acid-soluble glycoprotein, alpha(1)-Acid glycoprotein, ceruloplasmin, haptoglobin and c-reactive protein, in the pig. Veter. Immun. Immunopath. 51:377-385.

Eriksen, H. R., M. Olff, R. Murison and H. Ursin. 1999. The time dependent dimension in stress responses: relevance for survival and health. Psychiatry Res. 85:39-50.

Fisher, A. D., M. A. Crowe, P. O'Kiely, and W. J. Enright. 1997a. Growth, behaviour, adrenal and immune responses of finishing beef heifers housed on slatted floors at 1.5, 2.0, 2.5 or 3.0 m^2 space allowance. Live. Prod. Sci. 51:245-254.

Fisher, A. D., M. A. Crowe, E. M. O'Nuallain, M. L. Monaghan, D. J. Prendiville, P. O'Kiely, and W. J. Enright. 1997b. Effects of suppressing cortisol following castration of bull calves on adrenocorticotropic hormone, *in vitro* interferon-gamma production, leukocytes, acute phase proteins, growth and feed intake. J. Anim. Sci. 75:1899-1908.

Gunn, G. J. and A. W. Scott. 1998. Economic losses associated with calf pneumonia. Cattle Practice. 6(3):259.

Harris, J. R., and J. Markl. 1999. Keyhole Limpet Haemocyanin (KLH): a biomedical review. Micron. 30:597–623.

Henry, J. P. 1993. Biological basis of the stress responses. News Physiol. Sci. 8:69 –74.

Hickey, M. C., B. Earley and A. D. Fisher. 2003. The effect of floor type and space allowance on the welfare of finishing steers. Irish J. Ag. Food Res. (*in press*).

Lanier J. L., T. Grandin, R. D. Green, D. Avery, and K. McGee. 2000. The relationship between reaction to sudden, intermittent movements and sounds and temperament. J. Anim. Sci. 78:1223-1229.

Lay, D.C. Jr., T. H. Friend, C. L Bowers, K. K. Grissom, and O. C. Jenkins. 1992. A Comparative Physiological and Behavioral Study of Freeze and Hot - Iron Branding Using Dairy Cows. J. Anim. Sci. 70:1121-1125.

Lefcourt A.M., J. Bitman, S. Kahl, and D. L. Wood. 1993. Circadian and ultradian rhythms of peripheral cortisol concentration in lactating dairy cows. J. Dairy Sci.76:1757-1762.

Lefcourt, A. M., and T. H. Elasser. 1995. Adrenal responses of Angus x Hereford cattle to the stress of weaning. J. Anim. Sci. 73:2669-2676.

Lekeux, P. 1995. Bovine respiratory disease complex: A European perspective. Bovine Practitioner 29:71-75.

Lensink, B. J., X. Boivin, P. Pradel, P. Le Neindre, and I. Veissier. 2000. Reducing veal calves' reactivity to people by providing additional human contact. J. Anim. Sci. 78:879–884.

Mackenzie, A. M., M. Drennan, T. G. Rowan, J. B. Dixon, and S. D. Carter. 1997. Effect of transportation and weaning on humoral immune responses of calves. Res. Veter. Sci. 63:227-230.

Mason, W. A. 2000. Early developmental influences of experience on behaviour, temperament and stress. In : Biology of Animal Stress: Basic Principles and Implications For Animal Welfare. (eds. G.P. Morberg and J. A. Mench) CABI Publishing, Oxon, U. K. pp 269–289.

Minton, J. E., and F. Blecha. 1990. Effect of acute stressors on endocrinological and immunological functions in lambs. J. Anim. Sci. 68:3145-3151.

Minton, J. E., T. R. Coppinger, P. G. Reddy, W. C. Davis, and F. Blecha. 1992. Repeated restraint and isolation stress alters adrenal and lymphocyte functions and some leukocyte differentiation antigens in lambs. J. Anim. Sci. 70:1126–1135.

Minton, J. E. 1994. Function of the Hypotalmus-Pituitary-Adrenal Axis and the Sympathetic Nervous System in Models of Acute Stress in Domestic Farm Animals. J. Anim. Sci. 72:1891–1898.

O'Connor, T. M., D. J. O'Halloran and F. Shanahan. 2000. The stress response and the hypothalamic-pituitary-adrenal axis: from molecule to melancholia. Q. J. Med. 93: 323-333.

Phillips, W. A., P. E. Juniewicz, M. T. Zavy, and D. L. Von Tungeln. 1989. The effect of the stress of weaning and transport on white blood cell patterns and fibrinogen concentration of beef calves of different genotypes. Can. J. Anim. Sci. 69:333-340.

Pollock, J. M., T. G. Rowan, J. B. Dixon, S. D. Carter, and D. F. Kelly. 1991. Estimation of immunity in the developing calf: cellular and humoral responses to keyhole limpet haemocyanin. Veter. Immun. Immunop. 29:105–113.

Qui, Y., Y. Peng, and J. Wang. 1996. Immunoregulatory role of neurotransimitters. Adv. Neuroimmun. 6:223-231.

Rothel, J. S., S. L. Jones, L. A. Corner, J. C. Cox, and P. R. Wood. 1990. A sandwich enzyme immunoassay for bovine interferon-gamma and its use for the detection of tuberculosis in cattle. Aust. Vet. J. 67:134-137.

SAS. 1988. SAS/STAT Users guide (Release 6.03). SAS Inst. Inc., Cary, NC, USA.

Sevi, A., L. Taibi, M. Albenzio, A. Muscio, S. Dell Aquila, and F. Napolitano. 2001. Behavioural, adrenal, immune and productive responses of lactating ewes to regrouping and relocation. J Anim. Sci. 79:642-652.

Seyle, H. 1946. The general adaptation syndrome and the diseases of adaptation. J. Clin. Endocrinol. 6:117–230.

Skinner, J. G., R. A. Brown, and L. Roberts. 1991. Bovine haptoglobin response to clinically defined field conditions. Vet. Rec. 128:147-149.

Snedecor, G. W., and W. G. Cochran. 1989. Statistical methods. 8th ed. Iowa State University Press, Ames.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and Procedures of Statistics with Special Reference to Biological Sciences. McGraw Hill book Company Inc., New York.

Earley, B., and M. A. Crowe. 2002. Effects of ketoprofen alone or in combination with local anesthesia during the castration of bull claves on plasma cortisol, immunological, and inflammatory responses. J. Anim. Sci. 80:1044-1052.

Veissier, I., and P. Le Neindre. 1989. Weaning in calves: Its effect on social organisation. Appl. Anim. Behav. Sci. 24:43-54.

Veissier, I., P. Le Neindre, and J. P. Garel. 1990a. Decrease in cow-calf attachment after weaning. Behav. Proc. 21:195-195.

Veissier, I., D. Lamy, and P. Le Neindre. 1990b. Social behaviour in domestic beef cattle when yearling calves are left with the cows for the next calving. Appl. Anim. Sci. 27:193-200.

von Borell, E. H. 2001. The biology of stress and its application to livestock housing and transportation assessment. J. Ainm. Sci. 79:E260-E267

Wittum, T.E., C. R. Young, L.H. Stanker, D. D. Griffin, L. J. Perino and E. T. Littledike. 1996. Haptoglobin response to clinical respiratory tract disease in feedlot cattle. Am. J. Veter. Res. 57:646-649.

Wood, P. R., and H. F. Seow. 1996. T cell cytokines and disease prevention. Veter. Immun. Immunop. 54:33– 44.

Wood P. R., L.A. Corner, and P. Plackett. 1990. Development of a simple, rapid *in vitro* cellular assay for bovine tuberculosis based on the production of g interferon. Res. Vet. Sci. 49: 46-49.

Table 1. The effect of sampling time post weaning , sex and weaning on the temperament score of suckler calves

Temperament score [a]	Mean	Median	Minimum	Maximum	Significance
Sampling time					
6 h	3.1	2	1	12	ns
24 h	2.9	2	1	12	ns
48 h	3.0	2	1	8	ns
168 h	2.9	2	1	12	ns
Weaning					
Control	2.9	2	1	12	ns
Weaned	2.8	2	1	12	ns
Sex					
Male	2.6	2	1	8	ns
Female	3.3	2	1	12	ns

[a] Temperament score was calculated as described in materials and methods.

Table 2. The effect of time of sampling (T), weaning (W) and calf sex (S) on measures of plasma noradrenaline, adrenaline and cortisol concentration.

Item		**Male**		**Female**			**Statistical result**					
	Time	**Control**	**Wean**	**Control**	**Wean**		**T**	**S**	**W**	**T x S**	**T x W**	**S x W**
Noradrenaline	24	3.68	7.14	5.56	6.76	*F-value*	ns	ns	***	*	ns	*
(nmol/l)	48	4.32	8.02	5.98	6.58	*s.e.m.*	0.315	0.485	0.485	0.606	0.606	0.689
	168	3.89	8.6	3.59	5.16							
Adrenaline	24	1.20	2.30	1.26	1.60	*F-value*	ns	ns	ns	ns	ns	ns
(nmol/l)	48	1.50	1.93	1.27	1.62	*s.e.m.*	0.144	0.266	0.266	0.312	0.312	0.374
	168	1.40	1.31	1.20	1.55							
Cortisol	-168	6.8	7.3	7.0	8.7	*F-value*	***	ns	ns	ns	ns	ns
(ng/ml)	24	12.9	14.8	15.9	13.2	*s.e.m.*	0.51	0.73	0.73	0.96	0.96	1.03
	48	11.3	13.4	16.2	13.6							
	168	10.0	10.5	11.8	13.0							

Table 3. The effect of time of sampling (prior or 6h post weaning) and weaning (W) of bull calves only on measures of stress hormones, leukocyte profiles and *in vitro* interferon-gamma response to the novel mitogen concanavalin-A (Con-A) and antigen keyhole limpet haemocyanin (KLH)

Item	**Male**		**Statistical result**	
	Control	**Wean**	**W**	**s.e.m.**
Noradrenaline (nmol/l)	3.32	4.76	**	0.326
Adrenaline (nmol/l)	1.17	1.13	ns	0.213
Cortisol (ng/ml)	11.5	11.4	ns	1.52
Haptoglobin (g Hb binding capacity/ l)	0.16	0.21	ns	0.034
Fibrinogen (mg/100 ml)	487	589	ns	35.5
White blood cell (x 10^9 /l)	11.5	12.9	ns	0.567
% Neutrophil (N)	26.9	34.7	**	1.72
% Lymphocytes (L)	69.1	60.7	**	1.91
N : L ratio	0.40	0.62	**	0.050
Con-A[a]	0.70	0.50	ns	0.102
KLH[a]	0.38	0.01	ns	0.117

[a] Optical density value; absorbance at 450 nm

Table 4. The effect of time of sampling (T), weaning (W) and calf sex (S) on measures of the leukocyte population

Item	Time	Male		Female			Statistical result					
		Control	Wean	Control	Wean		T	S	W	T x S	T x W	S x W
White blood cell	-168	10.4	11.1	11.1	11.4							
(x 10^6/l)	24	12.0	12.7	11.4	12.2	*F-value*	***	ns	ns	ns	ns	ns
	48	12.0	11.9	11.7	12.1	*s.e.m.*	0.18	0.31	0.31	0.37	0.37	0.43
	168	11.7	10.8	11.4	11.7							
% Neutrophil (N)	-168	26.5	28.8	25.3	23.7							
	24	28.0	34.9	25.4	31.8	*F-value*	**	*	***	ns	**	†
	48	28.2	34.5	26.4	28.6	*s.e.m.*	1.02	1.06	1.06	1.64	1.64	1.49
	168	25.4	41.9	25.6	30.1							
% Lymphocyte (L)	-168	70.1	66.7	70.9	72.4							
	24	68.2	60.4	71.1	65.0	*F-value*	***	*	***	ns	*	ns
	48	67.7	60.6	69.4	67.5	*s.e.m.*	0.70	0.90	0.90	1.25	1.25	1.28
	168	70.9	64.4	70.3	66.2							
N : L ratio	-168	0.42	0.47	0.37	0.34	*F-value*	**	*	**	ns	†	ns
	24	0.44	0.61	0.36	0.51	*s.e.m.*	0.019	0.021	0.021	0.037	0.037	0.030
	48	0.44	0.59	0.39	0.44							
	168	0.36	0.47	0.37	0.46							

Table 5. The effect of time of sampling (T), weaning (W) and calf sex (S) on measures of the plasma haptoglobin concentration and *in vitro* interferon-gamma response to the novel mitogen concanavalin-A (Con-A) and antigen keyhole limpet haemocyanin (KLH)

Item		**Male**		**Female**			**Statistical result**					
	Time	**Control**	**Wean**	**Control**	**Wean**		**T**	**S**	**W**	**T x S**	**T x W**	**S x W**
Haptoglobin [a]	-168	0.17	0.35	0.19	0.31	*F-value*	ns	ns	ns	ns	ns	ns
	24	0.17	0.24	0.18	0.24	*s.e.m.*	0.312	0.04	0.04	0.056	0.056	0.057
	48	0.19	0.40	0.32	0.20							
	168	0.10	0.29	0.31	0.16							
Con-A[b]	-168	1.05	0.89	1.33	1.10	*F-value*	***	ns	ns	ns	ns	ns
	24	0.85	0.64	0.84	0.94	*s.e.m.*	0.058	0.077	0.077	0.105	0.105	0.108
	48	0.86	0.58	0.73	0.68							
	168	0.64	0.72	1.05	0.64							
KLH[b]	-168	0.48	0.32	0.51	0.42	*F-value*	***	ns	**	ns	ns	ns
	24	0.44	0.07	0.33	0.11	*s.e.m.*	0.033	0.062	0.062	0.073	0.073	0.087
	48	0.41	0.11	0.20	0.11							
	168	0.21	0.06	0.29	0.08							

[a] g Hb binding capacity/ l

[b] Optical density value; absorbance at 450 nm

Figure 1. The effect of sampling time, calf sex and treatment on calf plasma fibrinogen concentration.