

Study 3: An assessment of responsiveness of calves from the index farm, with known essential element deficiencies to selenium supplementation, May 2007 to April 2008

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Abstract

Long-term problems of cattle performance have been observed on a farm (the 'index farm') in Co. Kilkenny, Ireland. Earlier studies have indicated that selenium (Se) deficiency may be contributing to this problem. The aims of this study were to evaluate the efficacy of different forms of selenium supplementation to maintain the Se status and performance of animals, and to compare the performance of Se supplemented animals with un-supplemented animals moved to an unaffected farm. In a randomised controlled study Friesian cross calves (n = 42) were assigned to one of four groups; *Tx1OnFC* 2ml sc injection sterile water, remained on the index farm, n = 11. *Tx2BVP* sc injection BVP Barium Selenate LA® (1 mgSe/kg LBW), remained on the index farm, n = 10. *Tx3Vital* im injection of Vital® (68mgVit E/ml & 1.5mgSe/ml) at 2ml/45kg LBW, remained on the index farm, n = 11. *Tx4OffFC* 2ml sc injection of sterile water, animals remained on the index farm for 41 days, moved to unaffected farm for 125 days, subsequently returned to the index farm, n=9. All animals had normal Se and GPx concentrations at the start of the study on Day 0, however, Se and glutathione peroxidase GPx concentrations declined in all groups until Day 307 and Day 280, respectively. Se concentrations were below normal (120 µg/kg) in *Tx1OnFC* and *Tx3Vital* at 13 of the 21 sampling points, and in 7 of the 21 sampling point in *Tx4OffFC*, following their return to the index farm. *Tx2BVP* had higher Se and GPx concentrations compared with *Tx3Vital*, however, neither form of Se supplementation was sufficient to overcome the poor average daily gains (ADG) on the index farm. Following acclimatisation, *Tx4OffFC* animals, moved to the unaffected farm had improved ADG and normal Se concentrations, but both declined upon return to the index farm. The Se deficit status of the index farm was confirmed by the movement of animals off, and subsequently back to the index farm, confirming the localised nature of the deficiency. Selenium supplementation had a positive impact on blood Se status, but was not sufficient to overcome the shortfall in animal performance, suggesting that Se deficiency may be just a component of a more complex interaction. In support of this view, marginal to deficient concentrations of Cu and iodine (I) were also observed in all groups. The presence of radiodense growth retardation lines is consistent with poor calf growth, but does not provide any definitive insight into the cause of the problem. Two patterns of Cd excess were observed during this study: a large Cd peak and background Cd exposure. The significance of these findings is uncertain but subject to further study. Understanding the potential complex interaction between element excesses and deficiencies on the index farm could prove important.

1 Introduction

Shortfalls in the performance of cattle on a dairy farm in the south east region of Ireland - Castlecomer, Co. Kilkenny (subsequently referred to as the index farm) were identified. The problem principally presents as ill-thrift and stunted growth in growing cattle, although poor body condition and reduced milk yield in adult cattle has also been observed. Growing animals appear normally proportioned, but of small stature. Young growing animals (up to 2 years of age) and cows are predominately affected. Growing cattle are expected to achieve an average growth rate of 0.75 kg per day; however, weight gains on the index farm vary dramatically throughout growing periods. Periods of time have been identified when the majority of young animals on the farm are reported to exhibit very poor or negative growth rates. Both home bred and bought in cattle are affected, but once affected animals are removed from the index farm they achieve normal growth rates. These serious shortfalls in young animal performance occurring on the index farm are not explained by inadequate feed intake, management or housing issues (for a more detailed description see the *Overview*). Preliminary data from an investigation to elucidate the underlying mechanisms of the poor animal performance on the index farm indicated that a selenium (Se) deficiency may be a contributing factor to the ill thrift identified. Selenium responsive unthriftiness can occur at all ages in beef and dairy cattle, varying from sub clinical growth to sudden and rapid loss of condition usually associated with profuse diarrhea and in some case, a high mortality rate (Andrews *et al.*, 1968).

Selenium plays an important biological role in living organisms, principally through the incorporation of selenocystine into a family of proteins called selenoproteins (Stadtman, 1996). Only a few of the thirty or so identified mammalian selenoproteins have been functionally characterised (McKenzie *et al.*, 2002; Becket and Arthur, 2005). Selenoproteins are divided into at least three groups: (1) Six glutathione peroxidases (GPxs), responsible for reduction of hydroperoxides in cells, plasma and the gastrointestinal tract (Rotruck *et al.*, 1973). Of the six GPxs, all but two contain selenocystine (Herbette *et al.*, 2007); (2) Three iodothyroinine deiodinases (IDOs) responsible for metabolism of thyroid hormones. Selenocystine residues are present in the active centre of all three IDOs (Zagrodzki and Ratajczak, 2008). Severely Se depleted ruminants have decreased concentrations of T₃ and increased T₄ concentrations (Wichtel *et al.*, 1996); however, controlled depletion studies have demonstrated that Se is well retained by the brain, endocrine and reproductive organs (Beckett and Arthur, 2005) but lost rapidly from other tissue such as liver and muscle (Behne *et al.*,

1988). When the supply of Se is limited, the IDOs are better conserved than the GPxs; (3) Three thioredoxin reductases (TrxR), which reduce thioredoxin, are involved in many cell functions including cell growth, control of apoptosis and maintenance of cellular redox state, often through regulation of transcription factors (Rooke *et al.*, 2004). Importantly the fundamental cellular process, DNA synthesis, depends on the presence of Se within the catalytic site of TrxR (Arner and Holmgren, 2000).

Several clinical and sub clinical conditions in cattle respond to, or are prevented by, Se supplementation. The best known clinical form of Se deficiency is nutritional muscular dystrophy (white muscle disease) reported in lambs, calves (Waldner *et al.*, 1998; Ortman, 1999), foals and other species. The disease occurs primarily in young animals, clinical signs include stiff gait and unwillingness to move, affected muscles are often swollen and firmer and muscular tremors may occur. In a less acute form, however, Se deficiency interferes with the normal growth process (Foster, 2004; Hays and Swenson, 1993) and may result in a condition simply known as ill thrift, which has been identified in both sheep and cattle in Se deficient areas (Veling and Couston, 1995). This condition varies from sub clinical growth deficit to clinical unthriftiness with a rapid loss of weight and some mortality (Underwood and Suttle, 1999) and is similar to the losses reported on the index farm. In Australia and New Zealand, it has been well established that ill thrift in cattle and sheep can be controlled by Se supplementation (Andrews *et al.*, 1968; Underwood and Suttle, 1999).

Cattle dietary requirements for Se vary between 0.1 to 0.3 ppm (NRC, 2001, 2005) and the principal entry point of Se for animals is via plants, which in turn absorb inorganic forms from the soil. Globally, Se is widely distributed, with its availability limited by geological and climatic factors, and by farm management practices. In areas of Europe, New Zealand, North America and China, both low and high soil Se concentrations have been reported (Papp *et al.*, 2007; Witchel, 1998; Ortman, 1999). In Ireland, seleniferous soils are known to occur in small pockets of Meath, West Limerick, South Tipperary and North Dublin, and Se deficient soils occur in parts of Wexford, Cork, Tipperary, Waterford, East Galway and in Carlow (encompassing the Castlecomer plateau and the index farm; Fleming, 1978; McGrath and McCormack, 1999). Experimental data demonstrates that soil, herbage and blood Se concentrations are closely correlated (Andrews *et al.*, 1968) and Se deficiencies in Irish cattle are not uncommon. A survey of Irish abattoir cattle (n = 2,533), close to 11 % of animals were identified as having very low to low blood GPx (a proxy measure for Se) concentrations (Rogers, 2001).

The Se requirements of domestic livestock vary with the form of Se ingested (organic or inorganic), and with the nature of the rest of the diet, particularly its vitamin E and sulphur content, as well as variations in climatic and husbandry conditions. Combined supplements of Se and vitamin E have been more effective in raising antibody responses in animals when both nutrients are deficient, but are less effective in animals which have adequate concentrations of one or both nutrients (Finch and Turner, 1996). Vitamin E (a group of eight closely related compounds, with α -tocopherol the most biologically active and distributed) mainly functions in animals as a biological antioxidant; in association with GPxs and other vitamin and essential element containing enzymes, it protects cells against oxidative damage caused by free radicals. Selenium (a metalloid) has a strong tendency to complex with heavy metals such as cadmium (Cd) and mercury (Hg), and plays an important role in exerting a protective effect against the toxicity of both (Goyer, 1995; Underwood and Suttle, 1999). There is also some evidence that Se has an immunostimulatory effect; supplementing severely deficient dairy cows induced a self cure of sub-clinical mastitis (Ali-Vehmas, 1997) and Se deficiency in young calves was strongly associated with increased incidence of infectious diseases (Enjalbert *et al.*, 2006).

Selenium deficiencies were identified in earlier studies conducted on the index farm. Consequently the aims of this study were;

1. To evaluate the efficacy of different forms of selenium supplementation at maintaining the Se status, and performance of young animals on the index farm.
2. To compare the performance of Se supplemented calves on the index farm with un-supplemented calves, originating on the index farm, but moved to an unaffected farm (a farm with no history of Se deficiency or ill thrift) for a period of time.

The scope of this investigation was limited by two factors, only preliminary results were available upon which to base the study design, and young calves on the index farm were available only once annually, thereby limiting the time frame for access to these types on animals.

2 Materials and Methods

2.1 Study Overview

A randomised controlled field trial was conducted across two locations, the index farm (Co. Kilkenny, Ireland) and an 'unaffected' farm (Co. Meath, Ireland). Forty two Friesian cross calves sourced from the index farm, grouped by age, weight and sex were randomly assigned

to one of four treatment groups. The study was conducted over 363 days, from 02/05/2007 (Day 0) to 29/04/2008 (Day 363; Table 1). Blood samples were collected, and animals weighted on a fortnightly basis from Day 0 to Day 195 and subsequently on a monthly basis to the end of the study on Day 363. Initially all four groups remained on the index farm. One group was moved from the index farm on Day 41 to Day 166, and subsequently returned to the index farm until the end of the study. One calf died on Day 8 (10/05/07) and was therefore excluded from all analysis. The animal was found dead, this animal received no drug treatment prior to death and no post mortem was carried out. One calf died in a farm accident on Day 66 when it broke its neck in a drain on the farm, no post mortem was carried. A further two calves were identified for elective post mortem examination during the study; results from these calves were included in all analysis until their removal. All three animals were from the one treatment group, *Tx1OnFC*, calves that remained on the index farm for the duration of the trial and received no treatment.

Table 1. Sample number, sampling date and number of days relative to the start of the study for four groups of animals that either remained on the index farm from 02/05/07 to 29/04/08 and received either no treatment, *Tx1OnFC* (-●-); a selenium injection, *Tx2BVP* (-■-) or a selenium and vitamin E injection, *Tx3Vitasel* (-○-), or were moved off the index farm for a period of time, *Tx4OffFC* (-□-) from Day 41 to Day 166.

Sample number	Sampling date	Days relative to the start of the study
Bleed 1	02/05/2007	0
Bleed 2	15/05/2007	13
Bleed 3	29/05/2007	27
Bleed 4	12/06/2007	41
Bleed 5	26/06/2007	55
Bleed 6	10/07/2007	69
Bleed 7	24/07/2007	83
Bleed 8	07/08/2007	97
Bleed 9	20/08/2007	110
Bleed 10	04/09/2007	125
Bleed 11	18/09/2007	139
Bleed 12	02/10/2007	153
Bleed 13	16/10/2007	167
Bleed 14	30/10/2007	181
Bleed 15	13/11/2007	195
Bleed 16	11/12/2007	223
Bleed 17	08/01/2008	251
Bleed 18	06/02/2008	280
Bleed 19	04/03/2008	307
Bleed 20	02/04/2008	336
Bleed 21	29/04/2008	363

2.2 Selenium supplementation

Animal treatment (Tx) groups were as follows;

Tx1OnFC - No selenium supplementation, on farm control group. Calves received a subcutaneous injection of sterile water (2ml; water for injection) on Day 0 and remained on the index farm for the duration of the study. Mean calf age 73 ± 7.80 days and mean calf weight 64.9 ± 5.11 kgs, $n = 11$, reduced to $n = 10$ by Day 69 and further reduced to $n = 8$ by Day 202.

Tx2BVP - Selenium only, calves received a subcutaneous injection of BVP barium selenate LA® (Ballikskelligs Veterinary Products, Co. Kerry, Ireland; 50 mg Se/ml) injection on Day 0. Calves were injected at the upper limit of the recommended dosage of 1 mg Se/kg LBW. Mean calf age 69 ± 8.75 days and mean calf weight 62.9 ± 5.0 kgs, $n = 10$.

Tx3Vital - Selenium and vitamin E, calves received an intra muscular injection of Vital® (Norbrook Laboratories Ltd, Co. Down, N. Ireland), a white sterile emulsion with each ml containing 68 mg Vitamin E (dl-alpha-tocopheryl) acetate, 1.5 mg selenium (as potassium selenate) and 20 mg benzyl alcohol (as preservative). Calves were injected at the upper limit of the recommended dose of 2 ml per 45 kg LBW or 0.067 mg Se/kg LBW. Mean calf age 61 ± 9.16 days and mean calf weight 61.5 ± 5.76 kgs, $n = 11$.

Tx4OffFC - No selenium supplementation, off farm control group. Calves received one 2 ml subcutaneous injection of sterile water. Calves remained on the index farm for 41 days, were moved to the unaffected farm from Day 41 to Day 166 returned to the index farm for the remainder of the study (197 days). Mean calf age 65 ± 9.13 and mean calf weight 63.9 ± 5.79 kgs, $n = 9$.

Blood samples were collected from all animals before treatments were administered on Day 0. All animals were housed and fed indoors from Day 0 to Day 27, animals were at grass, either on the index farm or on the unaffected farm from Day 27 to Day 181, and finally animals were housed from Day 181 to the end on the study on Day 363. All housed animals were fed forage produced on the index farm and a proprietary calf ration (1 kg/calf/day). At and after first turnout to grass, all animals were routinely treated with a commercially available injectable ivermectin approximately every 6 weeks for the control of internal parasites (on four occasions) during the grazing season.

2.3 Assessment of animal health and performance

Calves were individually weighed fortnightly from Day 0 to Day 195 and subsequently on a monthly bases to the end of the study on Day 363. For the initial weighing, a calf crate was

used due to the size of the calves [mean calf weight per treatment group at the start of the experiment was *Tx1OnFC* 64.9 ± 5.4 kgs; *Tx2BVP* 62.9 ± 5.3 kgs; *Tx3Vitasel* 64.5 ± 6 kgs and *Tx4OffFC* 63.9 ± 6.2 kgs] and for all subsequent weighing a Tru-Test Indicator and aluminium platform (Tru-Test, O'Donovan Engineering, Co. Cork, Ireland) was used. For calves moved from the index farm (*Tx4OffFC*) to the unaffected farm, their weights were determined using an O'Donovan Engineering W100 indicator and aluminium platform (O'Donovan Engineering, Co. Cork, Ireland).

A visual clinical assessment of disease status was conducted on each animal at each weighing point. Animals were carefully observed to determine presence of normal demeanor and behaviors. External signs of clinical disease including nasal and ocular discharge, evidence of scour, poor gut fill, lameness, unusual swellings or asymmetry were noted. Coughing or increased respiratory effort was observed for on every occasion. Detailed hands-on clinical examinations (including auscultation and assessment of temperature) were not conducted.

2.3 Sample collection

Calves were blood sampled fortnightly from Day 0 to Day 195 and subsequently on a monthly basis to the end of the study on Day 363. Blood samples were collected, by jugular venipuncture into plain, lithium heparin, and EDTA blood tubes (BD Vacutainer Systems, Plymouth, UK). Sera was obtained after centrifugation of blood samples at $1,600 \times g$ for 20 min, following storage at room temperature for approximately 5 h (to facilitate transport to the laboratory) and at 4°C for 24 h; serum samples were submitted to the Clinical Pathology Laboratory, University Veterinary Hospital (UVH; University College Dublin, Ireland) for GPx analysis for all sampling periods, and for routine biochemistry panel from Day 167 to Day 363; or frozen at -20°C and stored. Whole blood (EDTA) samples were also submitted for complete blood counts (CBC) within 8 h of collection from Day 0 to Day 363. Whole blood (lithium heparin) samples were submitted to a commercial laboratory (Independent Analytical Services, Co. Carlow, Ireland) within 6 h of collection for mineral analysis from Day 0 to Day 363. Plasma samples were submitted on one occasion during the study on Day 167, to a commercial laboratory (Scottish Agricultural College Veterinary Services, Edinburgh, Scotland) to determine plasma inorganic iodine (PII) concentrations. Serum samples were also submitted once during the study, on Day 223, to a commercial laboratory (Eurofins, Co. Louth, Ireland) to determine fluoride concentrations.

2.4 Laboratory analysis

2.4.1 Glutathione peroxidase and biochemistry panel

Glutathione peroxidase activity was determined using the Randox RX imola™ Ransel kit (Randox Laboratories Ltd., Co. Antrim, N. Ireland). Samples were diluted 50 µl with 2 mls of diluting reagent prior to the start of the assays. Briefly, GPx catalysed the oxidation of glutathione by cumene hydroperoxidase. In the presence of glutathione reductase (GR) and NADPH, the oxidised glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP⁺. The decrease in absorbance at 340 nm was measured.

Other parameters measured were: Creatine kinase (CK), total protein (TP), albumin (Alb), urea, aspartate aminotransferase (AST), L-γ-glutamyltransferase (GGT), glucose (GLUC), non esterified fatty acids (NEFA) and beta-hydroxybutarate (BHB). All biochemical parameters were determined using the Randox RX imola™ assay kits (Randox Laboratories Ltd., Co. Antrim, N. Ireland) and were run in-house. Daily quality control (QC) samples were run for each assay on the Randox RX imola™, and results for QC were only accepted if they fell within one standard deviation of their expected value. Only if QC samples met their criteria could assays then be run.

2.4.2 Complete blood counts (CBC)

Samples were analysed using the Abbott CELL-DYN® 3500R (Abbott Laboratories Ireland Ltd., Citywest Business Campus, Dublin 24, Ireland) veterinary package. The CELL-DYN® 3500R uses flow cytometry to count and identify blood cells. Daily QC samples were run on the analyser to determine the performance of the machine and only if QC fell within expected parameters could the machine be used.

2.4.3 Whole blood mineral analysis

Whole blood (lithium heparin) samples were submitted to a commercial laboratory (Independent Analytical Services Ltd., (IAS), Bagenalstown, Co. Carlow, Ireland) within 5 h of collection for mineral analysis. Briefly, minerals in whole blood samples were dissolved in concentrated nitric acid during microwave digestion in a Milestone ETHOS EZ microwave system and APCU-TR40 pressure vessels using the following programme: sample size: 3.0 ml, acid: 7 ml concentrate HNO₃, ramp time: 15 min to 180 °C, hold time: 10 min at 180 °C, cooling: cooled to 50 °C (circa 1.5 hours). Digested samples were then made up to 25 ml with de-ionised water and filtered through a Whatman 540 filter paper. Samples were then

packaged and couriered to an accredited laboratory (INDIKATOR GmbH, D-42329 Wuppertal, Germany, <http://www.indikator-labor.de/>) for analyses using an Elan Inductively Coupled Plasma with a Mass Spectrograph Detector (ICP-MS) using certified standards. All standards and QCs were within normal range. All samples were analysed for Cd, selenium (Se) and sulphur (S), and for copper (Cu), iron (Fe), phosphorous (P), and zinc (Zn) from Day 41 to Day 363.

2.4.4 Plasma inorganic iodine - Day 167

Whole blood (lithium heparin) samples were submitted to a commercial laboratory on Day 167 (Scottish Agricultural College, <http://www.sac.ac.uk/consulting/>) for plasma inorganic iodine (PII) in all calves following the return of *Tx4OffFC* calves to the index farm on Day 166. Briefly, organic compounds were separated from plasma by precipitation and the resulting supernatant was subjected to cation exchange to remove any further traces of organic compounds. The eluted fractions were dried, ashed and re-suspended. Plasma Inorganic Iodine was measured using the Sendell-Kolthoss reaction involving the iodide catalysed reduction of cerium IV by arsenic III.

2.4.5 Serum fluoride – Day 223

Serum samples from all calves on Day 223 were submitted to a commercial laboratory (Eurofins Scientific Ireland, Laboratory Supplies, Co. Louth, Ireland) for fluoride using an ion selective electrode (ISE) method.

2.5 Post mortem examination

Two animals from *Tx1OnFC* control calves group, which remained on the index farm, were submitted for an elective post mortem examination (PM) during the study. Calf A (7.8 months, 196 Kg) was considered representative, based on live weight, of other animals in both the group (Day 195 *Tx1OnFC* LBW range 85 to 225 Kgs) and the overall study. However, Calf B (7.8 months, 85 Kg LBW 85 Kgs) was the lightest animal of the group, and neither representative of its group or other study animals. . Both animals were removed from the index farm on Day 202 and housed in isolation at the Department of Agriculture, Fisheries and Food (DAFF) farm (Abbotstown, Co. Dublin, Ireland). Animals were radiographed (see below) at the University Veterinary Hospital (UVH) and submitted for PM on the Day 208 (Calf A) and on the Day 210 (Calf B) to Dublin Regional Veterinary Laboratory (DAFF, Backweston, Co. Kildare, Ireland). Fresh liver, kidney, lung and adipose tissue samples were

submitted to a commercial laboratory (CAL Ltd., Co. Dublin, Ireland) within 24 h of collection for an elemental screen.

Rib and metatarsal bones from each animal were collected for ashing. Briefly, the flesh was stripped from the bones, then the bones were cut transversely using a band saw. Dry matter was determined by drying bone sections at 104°C for 24 h, bones were then ashed at 600°C for 8 h. Bone ash Ca and P were determined by spectrometry as previously described (Brady *et al.*, 2003) and samples were also submitted to a commercial laboratory (CAL Ltd., Co. Dublin, Ireland) for an extensive mineral analysis.

2.10 Radiology

The long bones of the two animals mentioned previously, and a further eight animals, (*Tx1OnFC* n = 1; *Tx2BVP* n = 2; *Tx3Vitasel* n = 3 and *Tx4OffFC* n = 2) were submitted for radiology examination at UVH in November 2007 and April 2008, respectively.

2.11 Statistical analysis

The average daily weight gain (ADG) was calculated from the live weight data. All data was validated by plotting the raw data for each outcome by time and animal to identify any unusual observations (extreme high or low values). These extreme values were then rechecked.

A repeated measures model using the MIXED procedure in SAS 9.1 (SAS Institute Inc. 2003 N.C, USA) was developed for each outcome measure. The time period between each sampling period was not equally spaced and this was accounted for in the model by selecting either a spatial correlation (power, Gaussian, linear or spherical), unstructured or compound symmetry structure to account for the correlation between measurements within the same animal. The appropriate correlation structure was chosen based on the AICC and BIC from the model. Trends over time by treatment were tested in a random coefficient model. When there was no significant trend, a repeated measures model was used.

Independent variables: The independent variables considered in each model included: treatment group, bleed number/date, animal sex and baseline measure taken at Bleed 1 (02/05/07). The baseline measure was included in the models to account for any pre-existing differences. The 2-way interaction between treatment and bleed number/date was tested. Where possible, the bleed number/date effect was modelled as a linear trend (initially for each

treatment). A backward selection procedure was used to eliminate terms from the model ($p > 0.05$). Residual and influence plots were used to assess the overall fit of the final model and to identify potential outliers. Outcome measures were appropriately transformed (e.g. log transformation) if the residual plots indicated that this was necessary.

Multiple outcomes: Testing multiple outcomes increases the possibility of making a type-I error. For example, when we have 15 outcome measures the probability of finding at least one significant by chance is $1 - (1 - 0.05)^{15} = 0.54$. To adjust for the increased possibility of making a type 1 error, the comparisons between means from the final models in each group of outcome measures were adjusted using the False Discovery Rate method (Benjamini and Hochberg, 1995) using the MULTTEST procedure in SAS. The mean comparisons considered from the final models were all those that involved the Treatment group. The weight and ADG were considered as two measurements of the same outcome and were not combined together when adjusting for multiple outcomes.

The health status, energy status, nutrition and growth models used for analysis are presented in the appendix, section 6.1.

3 Results

3.1 Growth: weight and ADG

An overview of mean group weights and average daily gains at significant points in time are presented in Table 2.

Mean group calf weights for the four treatment groups from Day 0 to Day 363 (21 sampling points) are presented in Figure 1.

Mean weight of *Tx1OnFC* control calves that remained on the index farm were lower (144.1 ± 13.17 ; 142.8 ± 13.33 kgs) on Day 153 and Day 167 compared with *Tx4OffFC* (170.78 ± 13.94 ; 171.0 ± 14.21 kgs) control calves that were moved off the index farm from Day 41 to Day 166.

Mean weight of *Tx3Vitasel* vitasel treated calves that remained on the index farm were lower (133.18 ± 13.45 ; 134 ± 12.58 kgs) on Day 153 and Day 167 compared with *Tx4OffFC* (170.78 ± 13.94 ; 171.0 ± 14.21 kgs) control calves that were moved off the index farm from Day 41 to Day 166.

Mean weight of *Tx2BVP* BVP treated calves that remained on the index farm were higher (103.8 ± 5.08 kgs) on Day 83 compared with *Tx4OffFC* (84.11 ± 8.15) control calves that were moved off the index farm from Day 41 to Day 166.

Mean group calf ADG for the four treatment groups at each of the 20 observed time periods are presented Figure 2.

Mean ADG in *Tx1OnFC* control calves that remained on the index farm were lower (0.53 ± 0.13 , 0.35 ± 0.09 ; 0.51 ± 0.09 ; 0.59 ± 0.06 kg/day) for the period ending on Day 97, 110, 139 and 153 compared with *Tx4OffFC* (1.01 ± 0.15 ; 0.73 ± 0.13 ; 1.62 ± 0.30 ; 1.45 ± 0.10 kg/day), control calves that were moved off the index farm from Day 41 to Day 166.

Mean ADG in *Tx1OnFC* control calves that remained on the index farm were higher (0.20 ± 0.07 , 1.02 ± 0.10 kg/day) period ending on Day 55 and Day 181 compared with *Tx4OffFC* (-0.12 ± 0.07 ; 0.47 ± 0.06 kg/day) control calves that were moved off the index farm from Day 41 to Day 166.

Mean ADG in *Tx2BVP* BVP treated calves that remained on the index farm were lower (0.14 ± 0.14 ; 0.59 ± 0.12 ; 0.64 ± 0.08 kg/day) for the period ending on Day 110, 139 and 153 compared with *Tx4OffFC* (1.01 ± 0.15 ; 0.73 ± 0.13 ; 1.62 ± 0.30 ; 1.45 ± 0.10 kg/day) control calves that were moved off the index farm from Day 41 to Day 166.

Conversely mean ADG in *Tx2BVP* BVP treated calves that remained on the index farm were higher (1.07 ± 0.07 kg/day) for the period ending on Day 181 compared with *Tx4OffFC* (0.47 ± 0.06 kg/day) control calves that were moved off the index farm from Day 41 to Day 166.

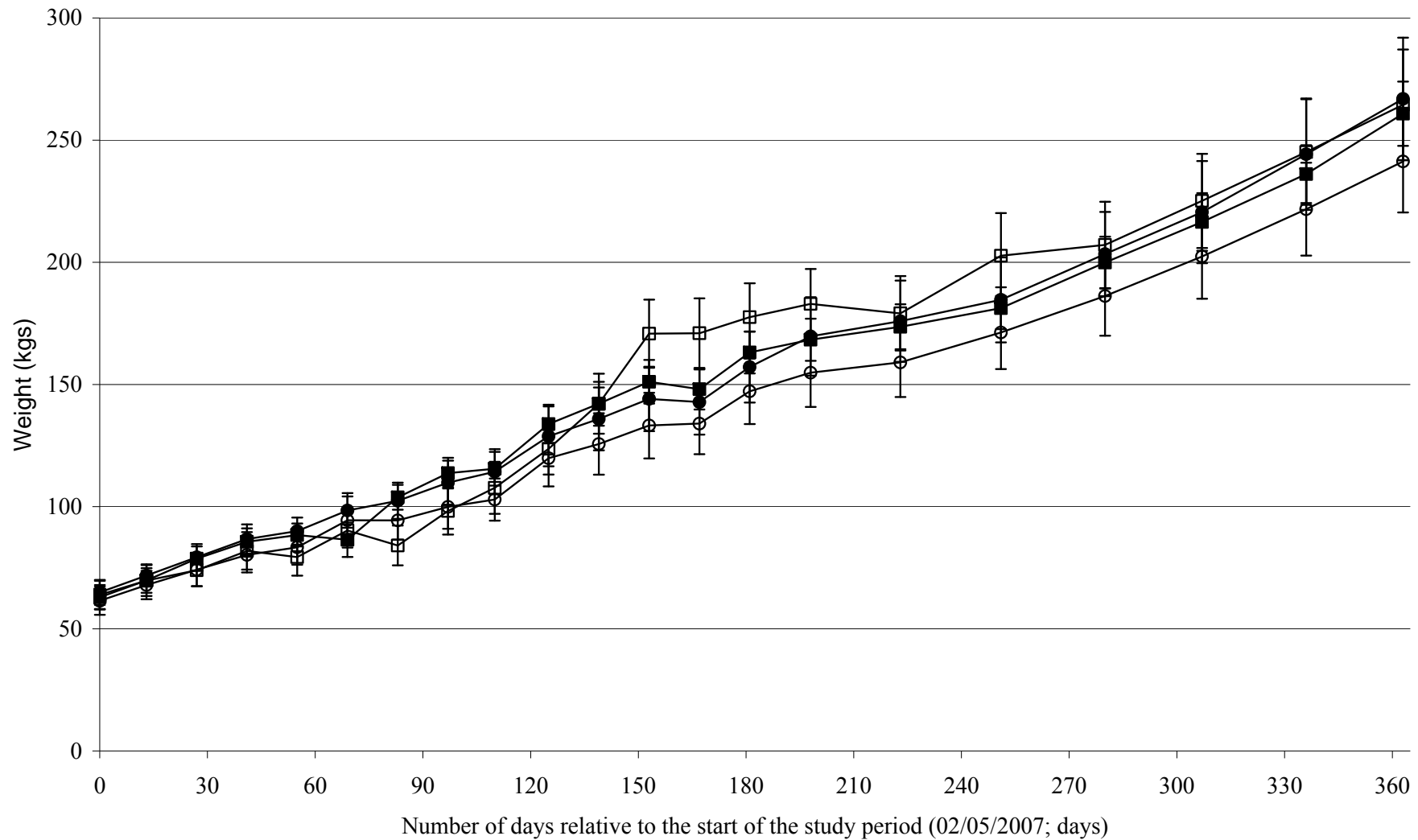
Mean ADG in *Tx3Vitalis* vitalis treated calves that remained on the index farm were lower (0.23 ± 0.1 ; 0.42 ± 0.16 ; 0.54 ± 0.09 kg/day) for the period ending on Day 110, 139 and 153 compared with *Tx4OffFC* (1.01 ± 0.15 ; 0.73 ± 0.13 ; 1.62 ± 0.30 ; 1.45 ± 0.10 kg/day) control calves that were moved off the index farm from Day 41 to Day 166.

Mean ADGs in *Tx3Vitalis* vitalis treated calves that remained on the index farm were higher (0.94 ± 0.09 kg/day) for the period ending on Day 181 compared with *Tx4OffFC* calves (0.47 ± 0.06 kg/day) control calves that were moved off the index farm from Day 41 to Day 166.

Table 2. Mean \pm SEM (range) live weight (kgs) and average daily gains (ADG, kg/day) for calves receiving either no treatment, *Tx1OnFC*; barium selenate injection, Tx 2 BVP; combined VitaminE/selenium injection, Tx3 Vitasel or calves moved off the index farm from 12/06/07 to 15/10/07, but receiving no other treatments, *Tx4OffFC* for the study period 02/05/07 to 29/04/08. ^{a,b} Within rows results with different superscripts are different $P < 0.05$

Live weight (kgs)			Treatment group			
Period of interest	Date	Day	<i>Tx1OnFC</i>	<i>Tx2BVP</i>	<i>Tx3 Vitasel</i>	<i>Tx4OffFC</i>
Start date	02/05/07	0	64.91 \pm 5.43 (52, 92)	62.90 \pm 5.0 (42, 90)	61.45 \pm 5.76 (39, 96)	63.89 \pm 5.79 (37, 93)
Turn out to pasture	29/05/07	27	79.27 \pm 5.43 (54, 105)	78.70 \pm 5.0 (57, 99)	74.18 \pm 6.69 (40, 107)	74.0 \pm 6.62 (42, 107)
<i>Tx4OffFC</i> leave index farm	12/06/07	41	86.73 \pm 6.0 (60, 114)	85.60 \pm 5.52 (61, 113)	80.27 \pm 7.20 (43, 113)	81.89 \pm 7.66 (52, 121)
Mid grazing	20/08/07	110	114.30 \pm 9.22 (66, 159)	155.5 \pm 6.90 (80, 162)	102.91 \pm 8.63 (62, 138)	107.67 \pm 10.64 (71, 157)
<i>Tx4OffFC</i> return to index farm	16/10/07	167	142.8 \pm 13.33 (75, 198) ^a	148.0 \pm 8.32 (96, 199) ^a	134.0 \pm 12.58 (67, 194) ^a	171.0 \pm 14.21 (111, 243) ^b
Winter housing	30/10/07	181	157.1 \pm 14.51 (84, 215)	163.1 \pm 8.60 (107, 213)	147.18 \pm 13.40 (74, 206)	177.56 \pm 13.85 (119, 247)
Mid winter housing	06/02/08	280	203.38 \pm 17.22 (147, 275)	199.9 \pm 10.61 (138, 262)	186.18 \pm 16.22 (89, 263)	207.11 \pm 17.71 (143, 292)
End date	29/04/08	363	266.88 \pm 25.08 (175, 367)	260.8 \pm 13.15 (191, 341)	241.27 \pm 20.91 (118, 346)	264.44 \pm 22.62 (184, 377)

ADG (kgs)			Treatment group			
Period of interest	Period ending	Days	<i>Tx1OnFC</i>	<i>Tx2BVP</i>	<i>Tx3 Vitasel</i>	<i>Tx4OffFC</i>
Start to turn out to pasture	29/05/07	0-27	0.53 \pm 0.08 (0.07, 1.04)	0.58 \pm 0.06 (0.33, 0.86)	0.47 \pm 0.07 (0.04, 0.79)	0.37 \pm 0.05 (0.19, 0.56)
<i>Tx4OffFC</i> leave index farm	12/06/07	27-41	0.53 \pm 0.08 (0.21, 1.0)	0.49 \pm 0.08 (0.14, 1.0)	0.44 \pm 0.07 (0.14, 0.93)	0.56 \pm 0.12 (0.0, 1.07)
Tx4 leave to mid grazing	20/08/07	41-110	0.38 \pm 0.05 (-0.09, 0.70)	0.43 \pm 0.05 (0.2, 0.71)	0.33 \pm 0.04 (0.04, 0.55)	0.37 \pm 0.06 (0.07, 0.62)
<i>Tx4OffFC</i> return to index farm	16/10/07	110-167	0.50 \pm 0.08 (0.16, 0.86) ^a	0.56 \pm 0.05 (0.28, 0.69) ^a	0.54 \pm 0.08 (0.08, 1.03) ^a	1.11 \pm 0.08 (0.70, 1.51) ^b
Tx4 return to housing	30/10/07	167-181	1.02 \pm 0.10 (0.57, 1.50) ^a	1.07 \pm 0.07 (0.79, 1.36) ^a	0.94 \pm 0.09 (0.50, 1.64) ^a	0.47 \pm 0.06 (0.29, 0.79) ^b
Calf turn out to housing	30/10/07	27-181	0.50 \pm 0.06 (0.19, 0.78)	0.55 \pm 0.04 (0.29, 0.75)	0.47 \pm 0.05 (0.14, 0.70)	0.67 \pm 0.06 (0.42, 0.96)
Mid winter housing	06/02/08	181-280	0.41 \pm 0.05 (0.19, 0.61)	0.37 \pm 0.04 (0.13, 0.49)	0.39 \pm 0.04 (0.15, 0.58)	0.31 \pm 0.05 (0.11, 0.52)
End	29/04/08	280-363	0.77 \pm 0.10 (0.34, 1.11)	0.73 \pm 0.04 (0.60, 0.95)	0.66 \pm 0.07 (0.35, 1.0)	0.69 \pm 0.06 (0.48, 1.02)
Start to end	29/04/08	0-363	0.55 \pm 0.05 (0.36, 0.76)	0.55 \pm 0.03 (0.4, 0.69)	0.50 \pm 0.06 (0.22, 0.72)	0.55 \pm 0.05 (0.39, 0.81)



1

Figure 1: Mean±SEM weight (kgs) for four groups of animals that either remained on the index farm from 02/05/07 to 29/04/08 and received either no treatment, Tx1OnFC (-●-); a selenium injection, Tx2BVP supplemented (-■-) or a selenium and vitamin E injection, Tx3Vital supplemented (-○-), or were moved off the index farm for a period of time, Tx4OffFC (-□-) from Day 41 to Day 166.

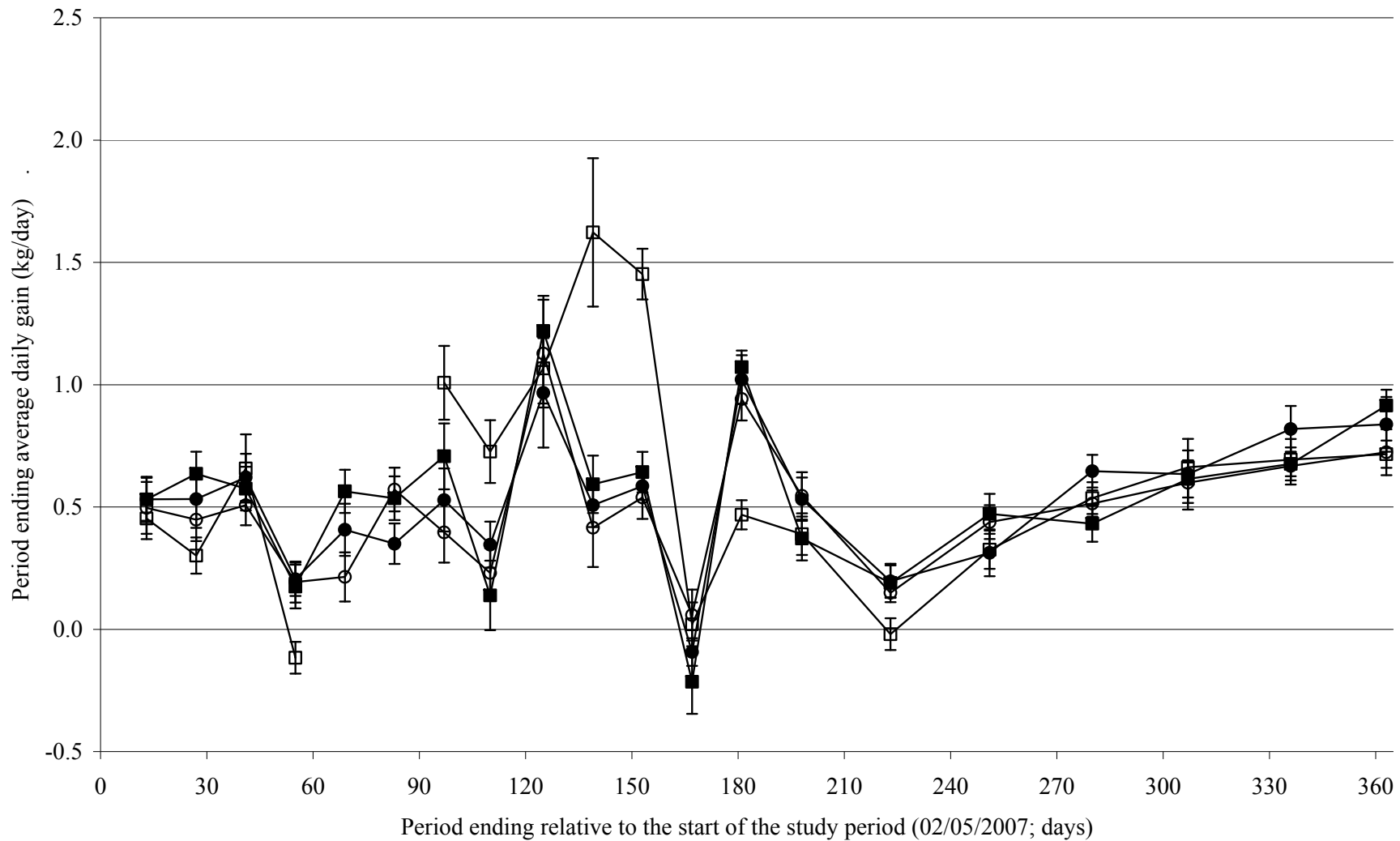


Figure 2: Mean±SEM average daily gain (ADG, kgs) for four groups of animals that either remained on the index farm from 02/05/07 to 29/04/08 and received either no treatment, *Tx1OnFC* (-●-); a selenium injection, *Tx2BVP* (-■-) or a selenium and vitamin E injection, *Tx3Vital* (-○-), or were moved off the index farm for a period of time, *Tx4OffFC* (-□-) from Day 41 to Day 166.

3.2 Health Status:

3.2.1 White Blood Cells:

Mean WBC for the four treatment groups from Day 0 to Day 363 (21 sampling points) are presented in the Appendix, section 2, Figure 2.1. There were very few differences in mean white blood counts (WBC).

Mean WBC in *Tx2BVP* BVP treated calves that remained on the index farm were lower ($6.98 \pm 0.51 \times 10^9/L$; $8.03 \pm 1.08 \times 10^9/L$) on Day 139 to Day 153 compared with *Tx4OffFC* ($11.47 \pm 0.94 \times 10^9/L$; $12.53 \pm 1.2 \times 10^9/L$) control calves that were moved off the index farm from Day 41 to Day 166.

Similarly mean WBC in *Tx3Vital* vitasel treated calves that remained on the index farm were lower ($6.97 \pm 0.71 \times 10^9/L$) on Day 153 compared with *Tx4OffFC* ($12.53 \pm 1.2 \times 10^9/L$) control calves that were moved off the index farm from Day 41 to Day 166.

Mean percentage lymphocytes for the four treatment groups from Day 0 to Day 363 (21 sampling points) are presented in the Appendix, section 2, Figure 2.2. There were no differences in percentage lymphocytes.

Mean percentage monocytes for the four treatment groups from Day 0 to Day 363 (21 sampling points) are presented in the Appendix, section 2, Figure 2.3.

Mean percentage monocytes in *Tx3Vital* vitasel treated calves that remained on the index farm were higher ($22.08 \pm 3.22 \%$) on Day 153 compared with *Tx4OffFC* ($11.22 \pm 1.09 \%$) control calves that were moved off the index farm from Day 41 to Day 166.

Mean percentage eosinophils for the four treatment groups from Day 0 to Day 363 (21 sampling points), are presented in the Appendix, section 2, Figure 2.4.

Mean percentage eosinophils in *Tx1OnFC* control calves that remained on the index farm were lower ($0.23 \pm 0.07 \%$) on Day 83 compared with *Tx4OffFC* ($1.24 \pm 0.33 \%$) control calves that were moved off the index farm from Day 41 to Day 166.

Mean percentage eosinophils in *Tx3Vital* vitasel treated calves that remained on the index farm were higher ($6.49 \pm 1.46 \%$) on Day 167 compared with *Tx4OffFC* ($0.89 \pm 0.47 \%$) control calves that were moved off the index farm from Day 41 to Day 166.

Mean percentage basophils for the four treatment groups from Day 0 to Day 363 (21 sampling points) are presented in the Appendix, section 2, Figure 2.5.

Mean percentage basophils in *Tx1OnFC* control calves that remained on the index farm were lower (0.40 ± 0.13 %) on Day 110 compared with *Tx4OffFC* (1.33 ± 0.19) control calves that were moved off the index farm from Day 41 to Day 166.

Mean percentage basophils in *Tx1OnFC* control calves that remained on the index farm were lower (0.54 ± 0.17 %) on Day 363 compared *Tx2BVP* (0.83 ± 0.11 %) BVP treated calves that remained on the index farm.

Mean percentage basophils in *Tx2BVP* BVP treated calves that remained on the index farm were lower (0.43 ± 0.07 ; 0.45 ± 0.11 ; 0.52 ± 0.16 and 0.57 ± 0.16 %) on Days 55, 83, 97 and 110 compared with *Tx4OffFC* (1.59 ± 0.64 ; 2.27 ± 0.84 ; 1.09 ± 0.22 and 1.33 ± 0.19 %) control calves that were moved off the index farm from Day 41 to Day 166.

Mean percentage neutrophils for the four treatment groups from Day 0 to Day 363 (21 sampling points) are presented in the Appendix, section 2, Figure 2.6.

Mean percentage neutrophils in *Tx1OnFC* control calves that remained on the index farm were lower (26.43 ± 3.14 %) on Day 153 compared with *Tx4OffFC* (49.65 ± 3.50 %) calves that were moved off the index farm from Day 41 to Day 166.

Mean percentage neutrophils in *Tx3Vitasel* vitasel treated calves that remained on the index farm were lower (27.65 ± 3.21 %) on Day 153 compared with *Tx4OffFC* (49.65 ± 3.50 %) control calves that were moved off the index farm from Day 41 to Day 166.

3.2.2 Red Blood Cells:

The mean number of red blood cells (RBC) was above the normal range in all four groups throughout the study. The mean mean red blood cell size (MCV) for all groups was below the normal range throughout the study. The mean amount of hemoglobin relative to the size of the cell (hemoglobin concentration) per red blood cell (MCHC) for all groups was below the normal range throughout the study, with the exception of one sampling period. Mean RBC, MCV and MCHC for the four treatment groups from Day 0 to Day 363 (21 sampling points) are presented in the Appendix, section 2, Figures 2.7, 2.10 and 2.12. There were no significant differences by treatment for RBC, MCV and MCHC. After adjusting for multiple comparisons there were no significant differences by treatment for packed cell volume (PCV) and mean cell hemoglobin (MCH), Appendix, section 2, Figures 2.8 and 2.11.

Mean haemoglobin concentrations for the four treatment groups from Day 0 to Day 363 (21 sampling points) are presented in the Appendix, section 2, Figure 2.9.

Mean haemoglobin concentrations in *Tx2BVP* BVP treated calves that remained on the index farm were higher (10.48 ± 0.27 ; 10.19 ± 0.19 g/dl) on Day 83 and Day 97 compared with *Tx4OffFC* (8.78 ± 0.28 ; 8.70 ± 0.27 g/dl) control calves that were moved off the index farm from Day 41 to Day 166.

Mean haemoglobin concentrations in *Tx3Vital* vitasel treated calves that remained on the index farm were lower (9.38 ± 0.41 g/dl) on Day 153 compared with *Tx4OffFC* (10.86 ± 0.21 g/dl) control calves that were moved off the index farm from Day 41 to Day 166.

3.2.3 Platelets:

Mean platelets were above the normal range for the first six sampling periods (Day 0 to Day 69) in all groups, but by Day 139 they fell within the normal range for all 4 groups until the end of the study on Day 363. Mean number of platelets for the four treatment groups from Day 0 to Day 363 (21 sampling points) are presented in the Appendix, section 2, Figure 2.13.

Mean number of platelets in *Tx1OnFC* control calves that remained on the index farm were lower (1046.73 ± 71.32 ; 1064.45 ± 31.09 and $1081 \pm 61.24 \times 10^9/L$) on Day 27, 41 and 55 compared with *Tx4OffFC* (1713.44 ± 363.04 ; 1638.0 ± 425.89 and $1718.78 \pm 400.76 \times 10^9/L$) control calves that were moved off the index farm from Day 41 to Day 166.

Mean number of platelets in *Tx2BVP* BVP treated calves that remained on the index farm were lower (1161.1 ± 64.1 ; 1190.4 ± 120.74 and $1171.7 \pm 88.10 \times 10^9/L$) on Day 27, 41 and 55 compared with *Tx4OffFC* (1713.44 ± 363.04 ; 1638.0 ± 425.89 and $1718.78 \pm 400.76 \times 10^9/L$) control calves that were moved off the index farm from Day 41 to Day 166.

Mean number of platelets in *Tx3Vital* vitasel treated calves that remained on the index farm were lower (1161.09 ± 119.17 ; $1229.36 \pm 134.97 \times 10^9/L$) on Day 27 and Day 55 compared with *Tx4OffFC* (1713.44 ± 363.04 ; $1718.78 \pm 400.76 \times 10^9/L$) control calves that were moved off the index farm from Day 41 to Day 166.

3.2.4 Energy Status

Mean urea and BHB concentrations for the four treatment groups from Day 167 to Day 363 (8 sampling points) are presented in the Appendix, section 4, Figures 4.4 and 4.8. Mean NEFA concentrations for the four treatment groups from Day 195 to Day 363 (7 sampling points) is presented in the Appendix, section 4, Figure 4.9. Urea, BHB and NEFA did not differ significantly by group.

3.2.5 Other

Mean TP, albumin, GGT and CK concentrations for the four treatment groups from Day 167 to Day 363 (8 sampling points) are presented in the Appendix, section 4, Figures 4.2, 4.3, 4.6 and 4.7. Total protein, albumin, GGT and CK did not differ by treatment and were within normal ranges.

Mean AST concentrations for the four treatment groups from Day 167 to Day 363 (8 sampling points) is presented in the Appendix, section 4, Figure 4.5.

Mean AST concentrations in *Tx1OnFC* control calves that remained on the index farm were higher (52.3 ± 3.8 iu/l) on Day 195 compared with *Tx4OffFC* (41.0 ± 1.65 iu/l) control calves that were moved off the index farm from Day 41 to Day 166.

Mean AST concentrations in *Tx3Vital* vitasel treated calves that remained on the index farm were higher (53.0 ± 3.21 iu/l) on Day 195 compared with *Tx4OffFC* (41.0 ± 1.65 iu/l) control calves that were moved off the index farm from Day 41 to Day 166.

3.3 Nutrition

3.3.1 Essential elements:

Sulphur, Zn, P and Fe did not differ by treatment but mean sulphur and phosphorous concentrations were above the normal range in all four groups across all time points measured. Mean S concentrations for the four treatment groups from Day 0 to Day 363 (21 sampling points) is presented in the Appendix, section 3, Figures 3.2. Mean Zn, P and Fe concentrations for the four treatment groups from Day 41 to Day 363 (13 sampling points) are presented in the Appendix, section 3, Figures 3.5, 3.6 and 3.7.

Mean Cu concentrations were below the normal level at all but two time points. Mean Cu concentrations for the four treatment groups from Day 41 to Day 363 (13 sampling points) is presented Figure 3.

Mean Cu concentrations in *Tx1OnFC* control calves that remained on the index farm were lower (894.3 ± 28.15 µg/kg) on Day 83 compared with *Tx4OffFC* (1107.44 ± 51.29 µg/kg) control calves that were moved off the index farm from Day 41 to Day 166.

Mean Cu concentrations in *Tx1OnFC* control calves that remained on the index farm were lower (795.13 ± 25.54 µg/kg) on Day 363 compared with *Tx3 Vital* (913.0 ± 53.39 µg/kg) vitasel treated calves that remained on the index farm.

Mean Cu concentrations in *Tx3Vital* vitasel treated calves that remained on the index farm were higher (862.82 ± 30.14 ; 1040.9 ± 62.09 µg/kg) on Day 139 and Day 223

compared with *Tx4OffFC* (714.89 ± 28.31 ; 890.44 ± 71.53 $\mu\text{g}/\text{kg}$) control calves that were moved off the index farm from Day 41 to Day 166.

Mean Cu concentrations in *Tx2BVP* BVP treated calves that remained on the index farm were lower (929.70 ± 34.78 $\mu\text{g}/\text{kg}$) on Day 83 but higher (838.7 ± 32.47 $\mu\text{g}/\text{kg}$) on Day 139 compared with *Tx4OffFC* (1107.4 ± 51.26 ; 714.89 ± 28.31 $\mu\text{g}/\text{kg}$) control calves that were moved off the index farm from Day 41 to Day 166.

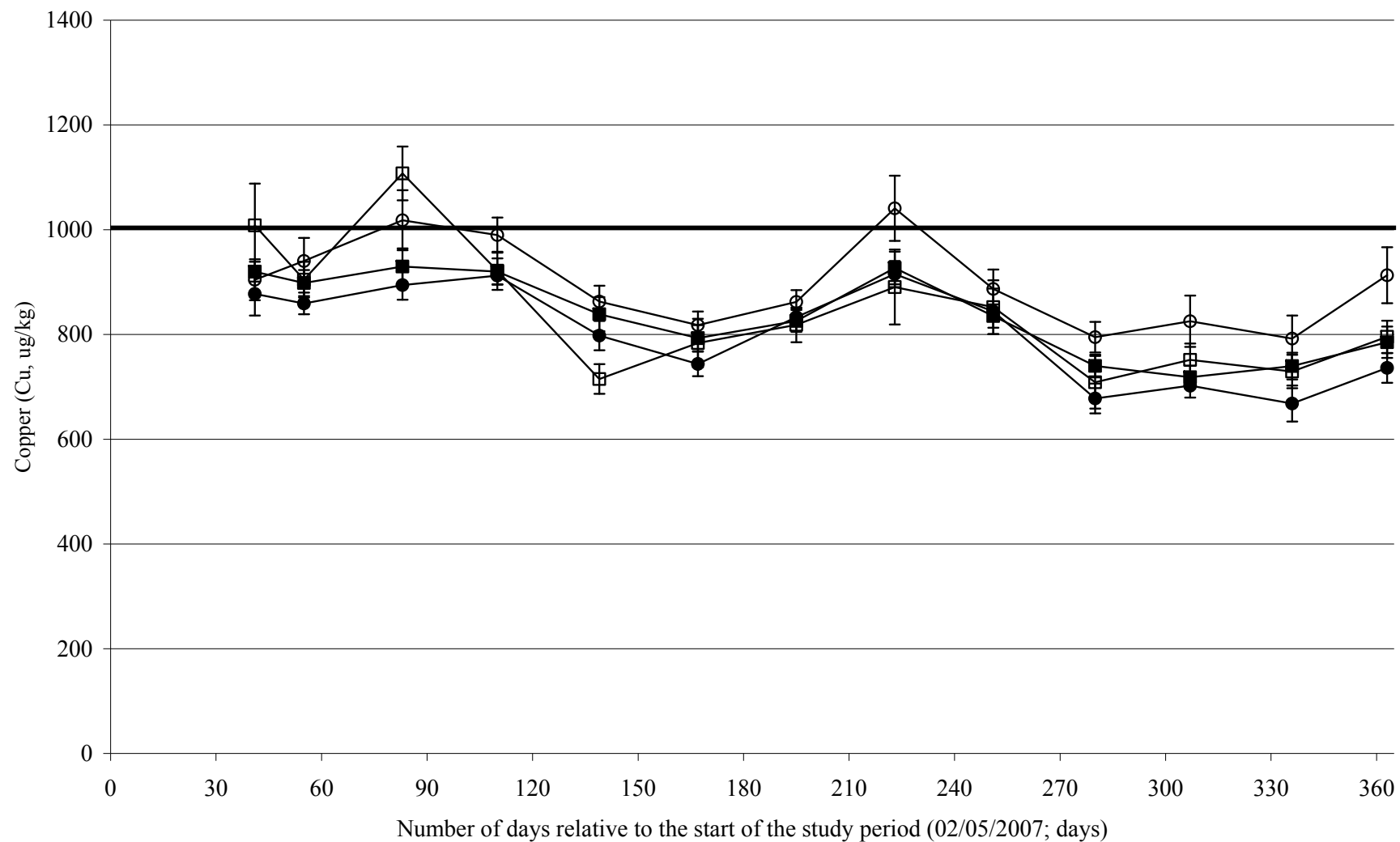


Figure 3: Mean±SEM whole blood copper (Cu, ug/kg) levels for four groups of animals that either remained on the index farm from 02/05/07 to 29/04/08 and received either no treatment, *Tx1OnFC* (-●-); a selenium injection, *Tx2BVP* (-■-) or a selenium and vitamin E injection, *Tx3Vitasel* (-○-), or were moved off the index farm for a period of time, *Tx4OffFC* (-□-) from Day 41 to Day 166. Normal Cu concentrations are above the solid black line.

Selenium concentrations were above the normal concentration (120 µg/kg) from Day 0 to Day 83 for all 4 groups. Selenium concentrations in *Tx1OnFC* control calves that remained on the index farm and *Tx3Vital* vitasel treated calves that remained on the index farm were below the normal concentration from Day 97 to Day 336. Selenium concentration in *Tx4OffFC* control calves that were moved off the index farm from Day 41 to Day 166 were below the normal concentration from Day 223 to Day 363, Figure 4.

Mean Se concentrations in *Tx1OnFC* control calves that remained on the index farm were lower (152.36 ± 3.79 ; 127.4 ± 8.61 ; 89.9 ± 8.13 ; 82.0 ± 6.34 ; 82.9 ± 8.09 ; 85.5 ± 11.18 ; 82.7 ± 11.58 ; 78.8 ± 8.24 ; 91.6 ± 8.94 µg/kg) on Day 41, 69, 97, 125, 139, 153, 167, 181 and 195 compared with *Tx4OffFC* (194.78 ± 10.93 ; 164.44 ± 8.16 ; 126.56 ± 4.91 ; 118.33 ± 8.75 , 128.56 ± 5.92 ; 128.78 ± 4.61 ; 124.33 ± 6.9 ; 119.67 ± 5.07 ; 127.0 ± 4.26 µg/kg) control calves that were moved off the index farm from Day 41 to Day 166.

Mean Se concentrations in *Tx1OnFC* control calves that remained on the index farm were lower (118.27 ± 3.33 ; 127.4 ± 8.61 ; 120.4 ± 8.57 ; 98.3 ± 10.26 ; 82.0 ± 6.34 ; 82.9 ± 8.09 ; 85.5 ± 11.18 ; 82.7 ± 11.58 ; 78.8 ± 8.24 ; 91.6 ± 8.94 ; 83.38 ± 13.82 ; 82.13 ± 10.43 µg/kg) on Days 55, 69, 83, 110, 125, 139, 153, 167, 181, 195, 223 and 251 compared with *Tx2BVP* (149.2 ± 4.37 ; 159.7 ± 5.93 ; 162.9 ± 6.89 , 125.1 ± 6.97 ; 121.6 ± 5.57 ; 118.9 ± 6.05 ; 117.80 ± 7.31 ; 120.6 ± 7.31 ; 117.4 ± 7.73 ; 148.9 ± 8.22 ; 128.8 ± 8.71 and 123.6 ± 7.80 µg/kg) BVP treated calves that remained on the index farm.

Mean Se concentrations in *Tx3Vital* vitasel treated calves that remained on the index farm were lower (122.09 ± 5.48 ; 133.55 ± 9.76 ; 93.55 ± 8.29 ; 86.73 ± 10.53 ; 83.73 ± 4.32 ; 96.91 ± 7.05 µg/kg) on Days 55, 83, 110, 251, 280 and 307 compared with *Tx2BVP* (149.2 ± 4.37 ; 162.9 ± 6.89 ; 125.1 ± 6.97 ; 123.6 ± 7.8 ; 109.2 ± 4.16 ; 123.8 ± 7.87 µg/kg) BVP treated calves that remained on the index farm.

Mean Se concentrations in *Tx3Vital* vitasel treated calves that remained on the index farm were lower (158.91 ± 7.70 ; 135.82 ± 10.44 ; 96.36 ± 9.5 ; 91.45 ± 9.88 ; 87.09 ± 8.71 ; 92.18 ± 8.97 ; 79.6 ± 5.06 µg/kg) on Day 41, 69, 97, 139, 153, 167 and 181 compared with *Tx4OffFC* (194.78 ± 10.93 ; 164.44 ± 8.16 ; 126.56 ± 4.91 ; 128.56 ± 5.92 ; 128.78 ± 4.61 ; 124.33 ± 6.9 ; 119.67 ± 5.07 µg/kg) control calves that were moved off the index farm from Day 41 to Day 166.

Mean Se concentrations in *Tx4OffFC* control calves that were moved off the index farm from Day 41 to Day 166 were lower (95.44 ± 7.68 ; 89.89 ± 6.76 ; 94.33 ± 7.68 ; 108.67 ± 11.47 µg/kg) on Days 223, 251, 307 and 336 compared with *Tx2BVP* (128.8 ± 8.71 ; 123.6 ± 7.8 ; 123.8 ± 7.87 ; 136.9 ± 9.42 µg/kg) BVP treated calves that remained on the index farm.

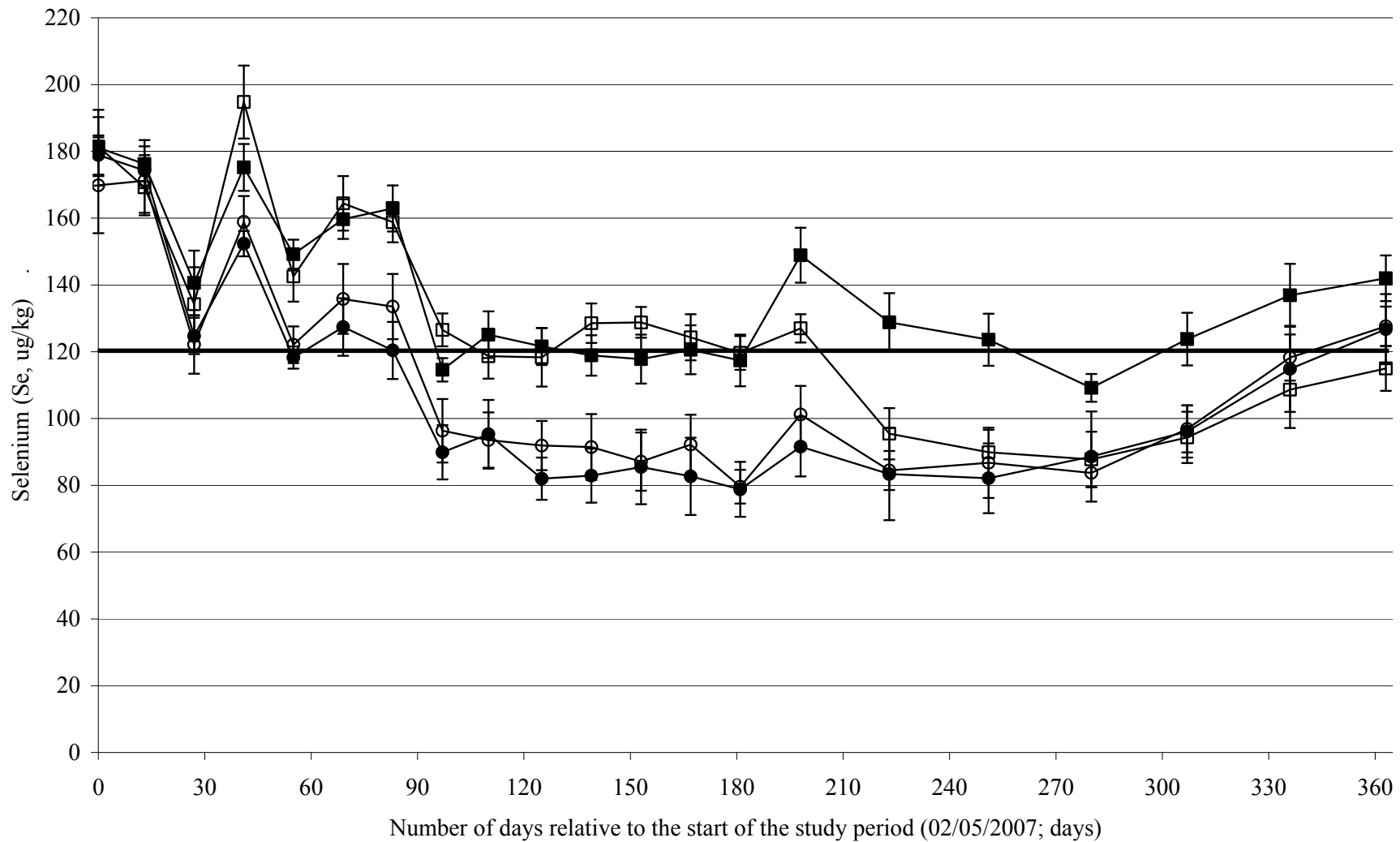


Figure 4: Mean±SEM whole blood selenium (Se, ug/kg) levels for four groups of animals that either remained on the index farm from 02/05/07 to 29/04/08 and received either no treatment, *Tx1OnFC* (-●-); a selenium injection, *Tx2BVP* (-■-) or a selenium and vitamin E injection, *Tx3Vitasel* (-○-), or were moved off the index farm for a period of time, *Tx4OffFC* (-□-) from Day 41 to Day 166. Normal Se concentrations are above the solid black line.

Mean GPx concentrations were above the normal range (40 U/ml PCV) for all 4 groups throughout the study period, Figure 5.

Mean GPx concentrations in *Tx1OnFC* control calves that remained on the index farm were lower (91.18 ± 5.53 ; 94.7 ± 7.81 ; 87.14 ± 9.63 ; 84.29 ± 12.34 ; 81.22 ± 12.27 ; 72.93 ± 12.96 ; 74.54 ± 13.63 ; 69.53 ± 13.27 ; 56.28 ± 11.54 ; 49.28 ± 9.47 U/ml PCV) on from Day 69 to Day 195 compared with *Tx4OffFC* (120.91 ± 6.8 ; 125.47 ± 6.05 ; 129.27 ± 5.46 ; 120.2 ± 4.22 ; 109.05 ± 4.43 ; 99.6 ± 3.37 ; 107.78 ± 3.41 ; 107.26 ± 4.13 ; 86.42 ± 3.64 ; 78.49 ± 3.79 U/ml PCV) control calves that were moved off the index farm from Day 41 to Day 166.

Mean GPx concentrations in *Tx1OnFC* control calves that remained on the index farm were lower (91.18 ± 5.53 ; 94.7 ± 7.81 ; 87.14 ± 9.63 ; 84.29 ± 12.34 ; 81.22 ± 12.27 ; 72.93 ± 12.96 ; 74.54 ± 13.63 ; 69.53 ± 13.27 ; 56.28 ± 11.54 ; 49.28 ± 9.47 ; 45.53 ± 10.62 ; 54.7 ± 11.33 ; 72.48 ± 12.06 ; 79.95 ± 8.69 ; 98.05 ± 8.38 U/ml PCV) from Day 69 to Day 336 compared with *Tx2BVP* (113.18 ± 4.01 ; 129.3 ± 4.34 ; 124.65 ± 5.39 ; 126.79 ± 4.62 ; 122.15 ± 4.06 ; 109.33 ± 6.03 ; 122.38 ± 6.8 ; 117.8 ± 8.7 ; 98.14 ± 7.97 ; 91.43 ± 7.21 ; 86.54 ± 7.05 ; 91.64 ± 6.49 ; 116.14 ± 8.74 ; 110.15 ± 5.58 ; 123.35 ± 4.79 U/ml PCV) BVP treated calves that remained on the index farm.

Mean GPx concentrations in *Tx3Vitasel* vitasel treated calves that remained on the index farm were lower (103.36 ± 7.58 ; 93.53 ± 9.01 ; 90.99 ± 9.71 ; 90.58 ± 11.8 ; 80.4 ± 12.36 ; 82.71 ± 11.42 ; 74.73 ± 10.72 ; 59.64 ± 8.34 ; 53.01 ± 6.94 ; 49.03 ± 6.04 ; 53.30 ± 5.24 ; 70.92 ± 5.30 ; 75.11 ± 4.15 ; 90.94 ± 4.08 ; 96.43 ± 4.40 U/ml PCV) on Day 83 to Day 363 compared with *Tx2BVP* (129.3 ± 4.34 ; 124.65 ± 5.39 ; 126.79 ± 4.62 ; 122.15 ± 4.06 ; 109.33 ± 6.03 ; 122.38 ± 6.8 ; 117.8 ± 8.7 ; 98.14 ± 7.97 ; 91.43 ± 7.21 ; 86.54 ± 7.05 ; 91.64 ± 6.49 ; 116.14 ± 8.74 ; 110.15 ± 5.58 ; 123.35 ± 4.79 ; 126.21 ± 4.92 U/ml PCV) BVP treated calves that remained on the index farm.

Mean GPx concentrations in *Tx3Vitasel* vitasel treated calves that remained on the index farm were lower (93.53 ± 9.01 ; 90.99 ± 9.71 ; 74.73 ± 10.72 ; 59.64 ± 8.34 U/ml PCV) on Days 97, 110, 167 and 181 compared with *Tx4OffFC* (129.27 ± 5.46 ; 120.2 ± 4.22 ; 107.26 ± 4.13 ; 86.42 ± 3.64 U/ml PCV) control calves that were moved off the index farm from Day 41 to Day 166.

Mean GPx concentrations in *Tx4OffFC* control calves that were moved off the index farm from Day 41 to Day 166 were lower (63.86 ± 5.24 ; 73.64 ± 7.66 ; 75.34 ± 7.35 ; 86.41 ± 6.74 ; 95.53 ± 5.58 U/ml PCV) on Day 251 to Day 363 compared with *Tx2BVP* (91.64 ± 6.049 ; 116.14 ± 8.74 ; 110.15 ± 5.58 ; 123.35 ± 4.79 ; 126.21 ± 4.92 U/ml PCV) BVP treated calves that remained on the index farm.

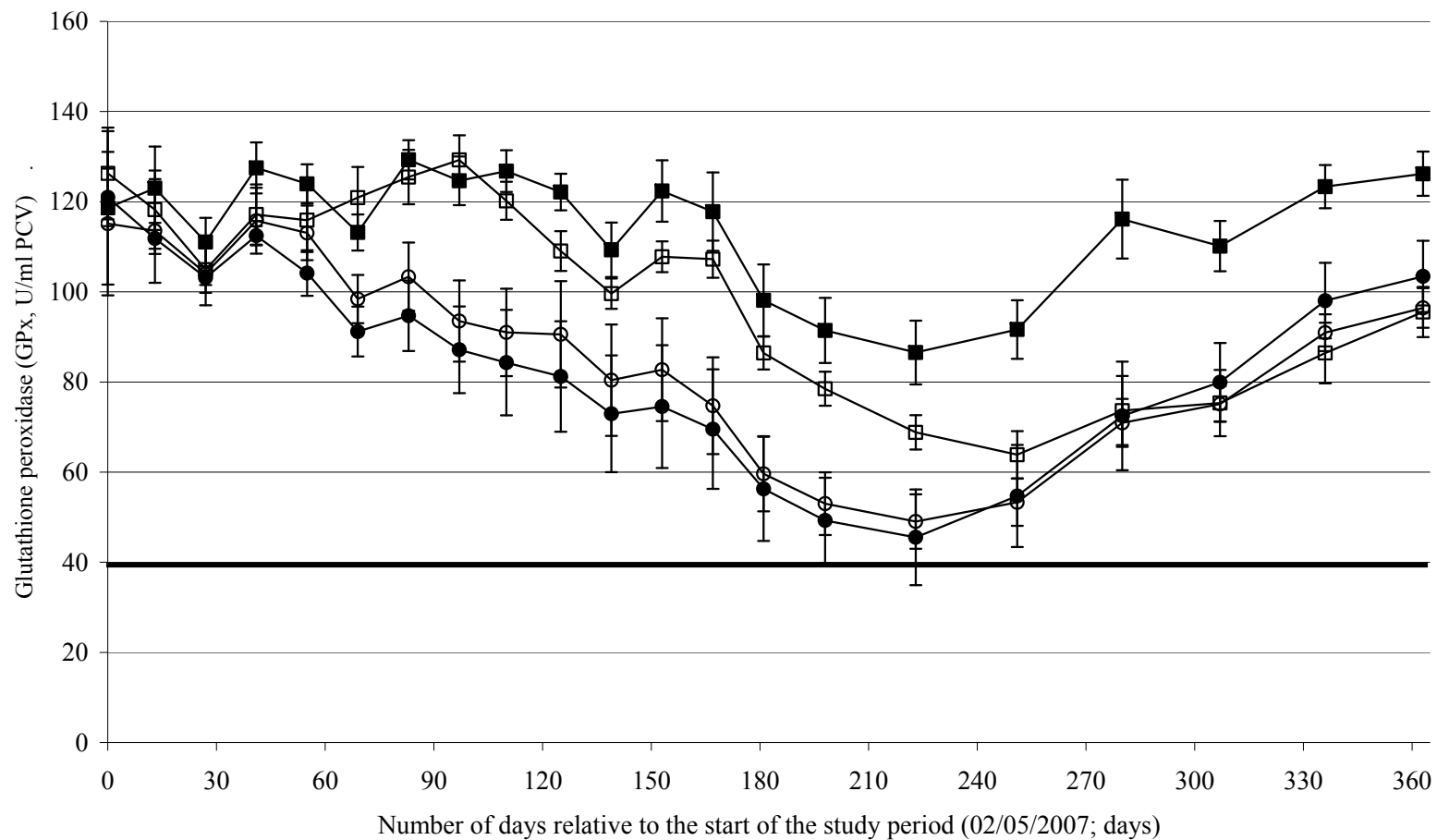


Figure 5: Mean±SEM whole blood glutathione peroxidase (GPx, U/ml PCV) for four groups of animals that either remained on the index farm from 02/05/07 to 29/04/08 and received either no treatment, *Tx1OnFC* (-●-); a selenium injection, *Tx2BVP* (-■-) or a selenium and vitamin E injection, *Tx3Vitasel* (-○-), or were moved off the index farm for a period of time, *Tx4OffFC* (-□-) from Day 41 to Day 166. Normal GPx concentrations are above the solid black line.

Mean group plasma inorganic iodine (PII) concentrations ($\mu\text{g/L}$), measured on Day 167 in all four treatment groups were below the normal range (101 to 300 $\mu\text{g/L}$), Figure 6. Plasma inorganic iodine concentrations were higher in *Tx1OnFC* ($77.2 \pm 11.13 \mu\text{g/L}$), *Tx2BVP* ($76.4 \pm 18.36 \mu\text{g/L}$) and *Tx3Vitasel* ($88.09 \pm 11.23 \mu\text{g/L}$) compared with *Tx4OffFC* ($19.44 \pm 1.04 \mu\text{g/L}$). Only nine of the forty animals sampled had PII concentrations within the normal range (101-300 $\mu\text{g/L}$, Rogers, 2001).

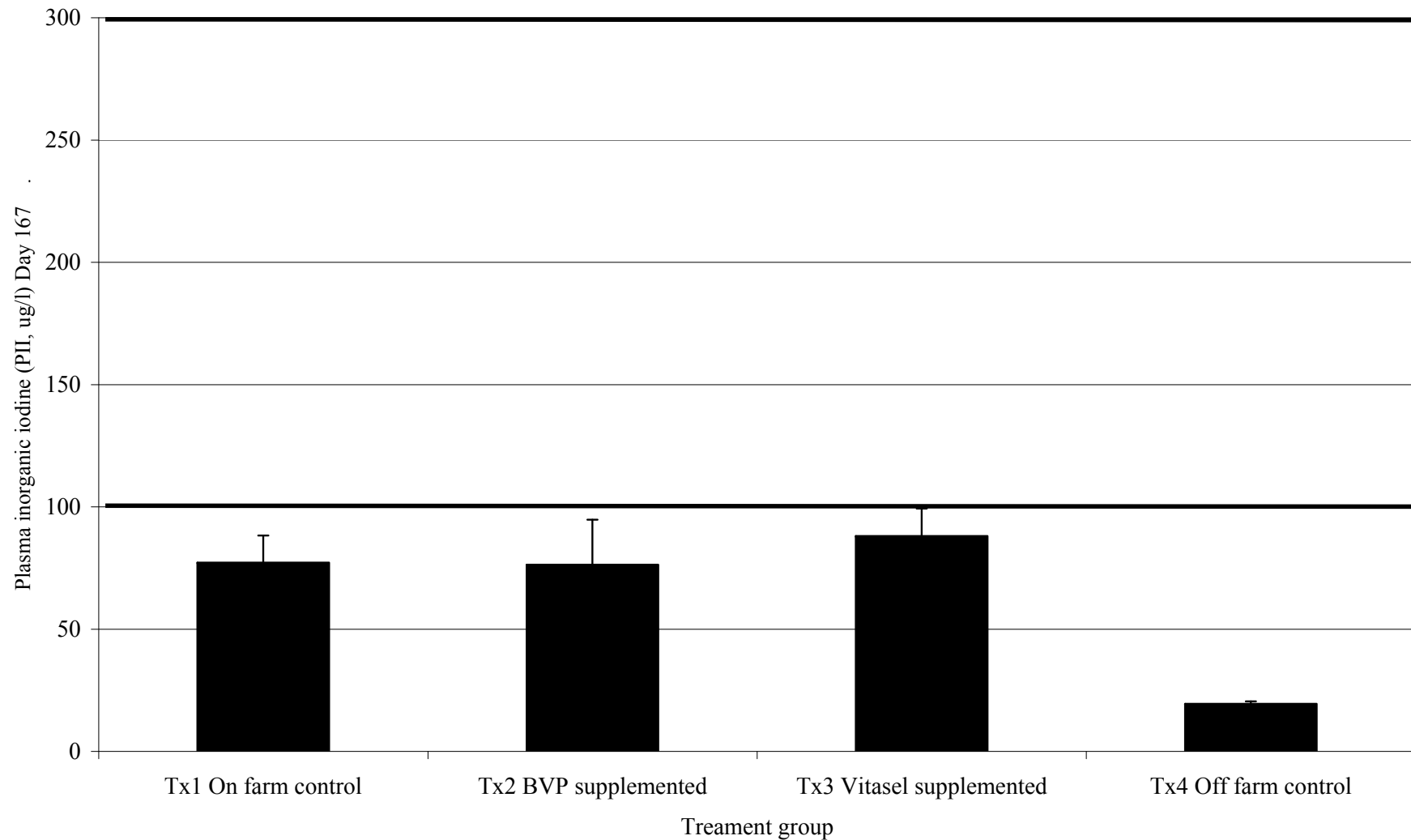


Figure 6. Mean±SEM plasma inorganic iodine (PII, ug/l) levels for four groups of animals that either remained on the index farm from 02/05/07 to 29/04/08 and received either no treatment, *Tx1OnFC* (-●-); a selenium injection, *Tx2BVP* supplemented (-■-) or a selenium and vitamin E injection, *Tx3Vitasel* supplemented (-○-), or were moved off the index farm for a period of time, *Tx4OffFC* (-□-) from 12/06/07 to 15/10/07. PII was measured on 16/10/07, the day after *Tx4OffFC* group returned to the index farm. The normal range is indicated by the solid black lines.

3.3.2 Heavy metals

Blood Cd concentrations did not differ by treatment, but there was a sharp rise in Cd concentrations on Day 83, 97 and 110 (Figure 7a). A further graph of the Cd results is presented in Figure 7b, following the exclusion of Cd results from Day 83, 97 and 110.

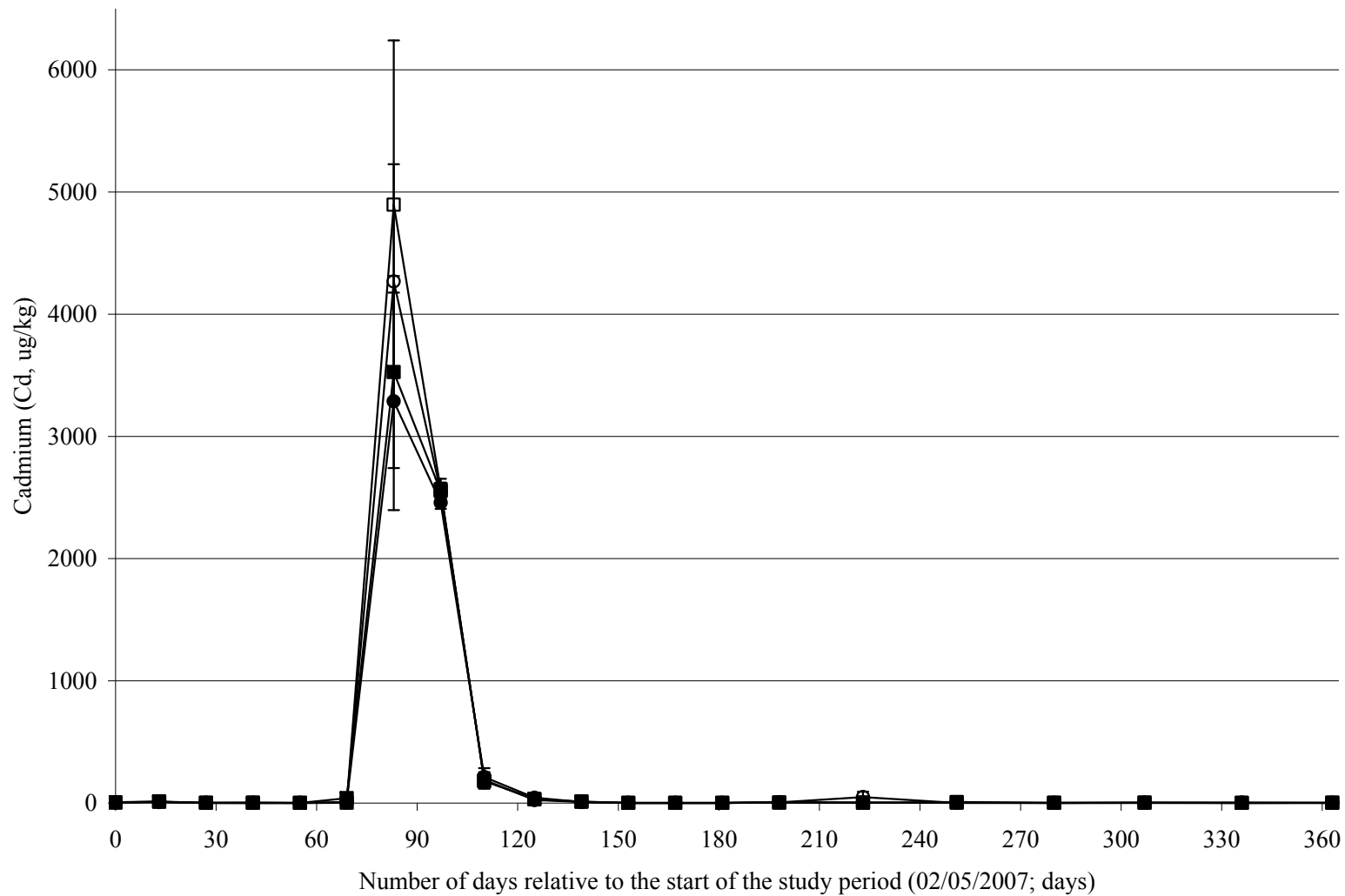


Figure 7a: Mean±SEM whole blood cadmium (Cd, ug/kg) levels for four groups of animals that either remained on the index farm from 02/05/07 to 29/04/08 and received either no treatment, *Tx1OnFC* (-●-); a selenium injection, *Tx2BVP* (-■-) or a selenium and vitamin E injection, *Tx3Vital* (-○-), or were moved off the index farm for a period of time, *Tx4OffFC* (-□-) from Day 41 to Day 166.

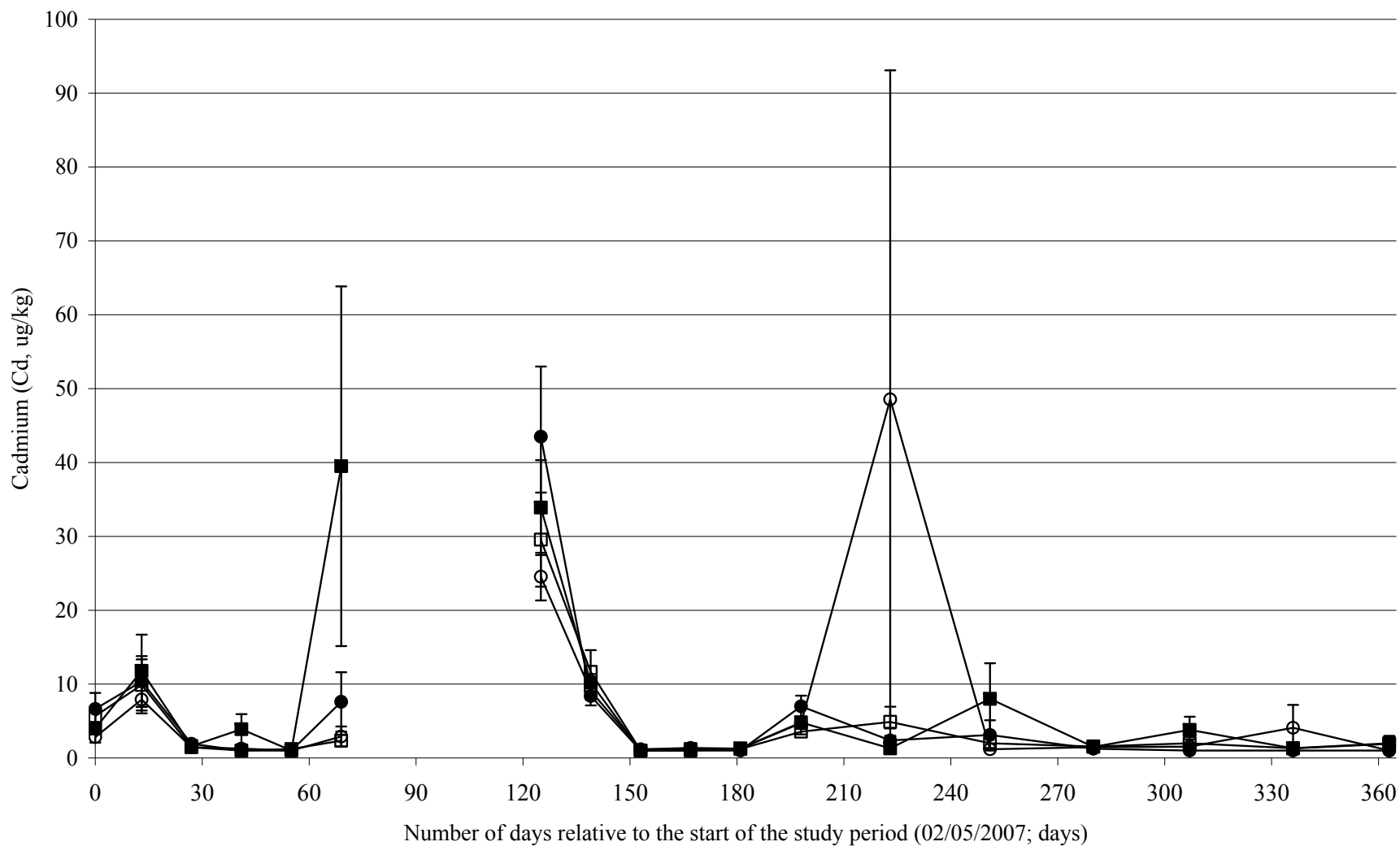


Figure 7b: Mean±SEM whole blood cadmium (Cd, ug/kg) levels for four groups of animals that either remained on the index farm from 02/05/07 to 29/04/08 and received either no treatment, *Tx1OnFC* (-●-); a selenium injection, *Tx2BVP* (-■-) or a selenium and vitamin E injection, *Tx3Vitasel* (-○-), or were moved off the index farm for a period of time, *Tx4OffFC* (-□-) from Day 41 to Day 166. Data removed from 3 time points, 24/07/07, 07/08/07 and 20/08/07 peak Cd levels.

3.4 Post mortem results

Both animals were in poor body condition as the time of PM; calf A had a long coarse coat, and calf B had ringworm. The following post mortem findings were noted:

- Calf A: chronic lymphocytic and eosinophilic cholangiohepatitis was observed in the liver associated with a light liver fluke infection (n = 5 fluke were isolated from the bile ducts). Villous atrophy was apparent in the intestines (Figure 8), along with a small number of coccidia. There was evidence of ostertagiosis in the abomasum with nematode larvae observed in the glands, which also contained fungal larvae, most likely secondary to achlorhydria. There was no evidence of nutritional myopathy.
- Calf B: had severe bronchopneumonia, and granulomatous oesophagitis and eosinophilic granulomatous reticulitis, the cause of which was unknown. Villous atrophy was apparent in the intestines (Figure 8), along with a small number of coccidia. Calf B also had a small number of trichostrongyles in its intestine. There was no evidence of nutritional myopathy.

RBCs were slightly raised in both animals (Calf A, 8.9×10^{12} ; Calf B, 8.84×10^{12}) at PM. Serum GGT was raised in Calf A (25 iu/L). Serum TP (65.7 g/L) and ALB (25.4 g/L) were low in Calf B. PII were low in both calves (Calf A; 17.0 $\mu\text{g/ml}$. Calf B; 37 $\mu\text{g/ml}$). Results of an elemental screen of liver, kidney, lung and adipose tissue samples are presented in the appendix, section 5, Table 5.1.

Results of elemental screens for bone ash are presented in the appendix, section 5, Table 5.2. The calcium:phosphorus (Ca:P) ratios for each bone type were within normal parameters. Calf A Ca:P for the metatarsal and rib bone were 2.07 and 1.99, respectively. Calf B Ca:P for the metatarsal and rib bone were 2.05 and 1.98, respectively. There is little variation in the Ca:P ratio in bone, being 2:1 in older animals and somewhat less in young animals (Doxey, 1971; Doyle, 1979).

3.5 Radiology results

One of the two animals submitted to PM in November 2007, Calf B, displayed horizontal radio-opaque sclerotic lines paralleling the physes of the right tibia in the metaphyseal region, these were growth retardation lines. Following PM, these bones were re x-rayed and growth retardation lines confirmed. Five of the eight additional animals submitted for radiology in April 2008 had confirmed radiodense growth retardation lines in either a radius or tibia.

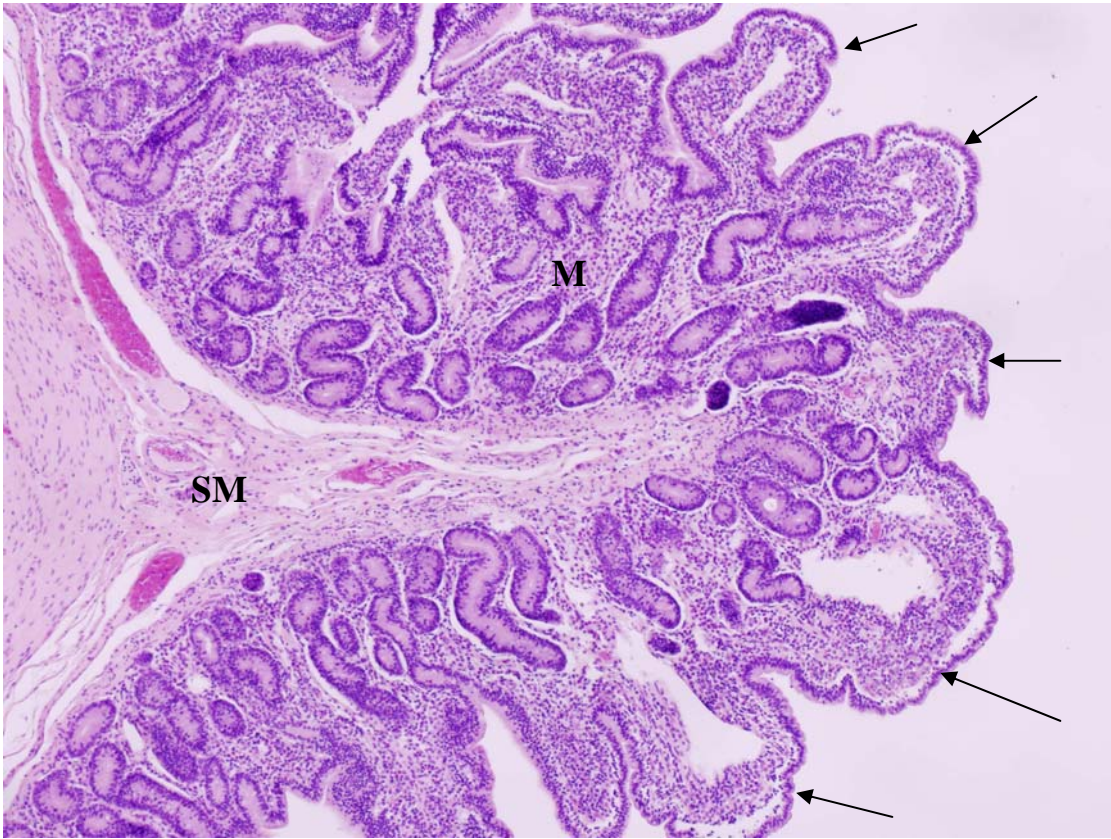


Figure 8: H&E section showing villous atrophy (→) in the intestine (smooth muscle, SM and mucosa, M) of calf A from *Tx1OnFC* control calves that remained on the index farm that was submitted for an elective post mortem in Nov 2007. Magnification 40x.

3.6 Animal health

On Day 153, pneumonia was diagnosed in one animal on the index farm. The farmer's veterinary surgeon was contacted, and the animal was treated with Nuflor® (Intervet, The Netherlands) for two days. In addition, all study calves on the index farm were treated with a chlortetracycline (CTC) powder in their feed for 5 days. On Day 154, all study calves on the index farm were vaccinated with Bovipast RSV (Intervet, The Netherlands; 4 mls) and Bovilis IBR marker (Intervet, The Netherlands; 2 mls). For consistency, animals that had been removed from the index farm to the unaffected farm in Co. Meath (*Tx4OffFC*) were also vaccinated on Day 156. On Day 153, the mean±SEM (min, max) white blood cells (WBC) counts for all three groups of animals on the index farm and for the group on the unaffected farm in Co. Meath was 7.66±0.44 (2.98, 15.0) and 12.53±1.20 (6.69, 18.6), respectively.

4 Discussion

Calves are typically born with adequate Se (Quigley and Drewry, 1998), and mean Se and GPx concentrations were similar, and at normal concentrations in all four groups of animals at the start of this study. By Day 55 and Day 69, Se and GPx concentrations, respectively, were higher in *Tx2BVP* animals, the Se only supplemented group, compared with *Tx1OnFC* (receiving no treatment), however, neither group were considered Se or GPx deficient. Selenium and GPx concentrations were also higher in *Tx2BVP*, the Se only supplemented group, by Day 55 and Day 83, respectively, compared with *Tx3Vital*, the Se/VitE supplemented group, again, however, neither group were considered either Se or GPx deficient, by this time. A time delay from Se supplementation to the increase in whole blood Se concentrations is not unusual, a complete response to Se supplementation in whole blood Se requires a time span equal to the average life span of the red blood cells, which in cattle is approximately 90 to 128 days (Stowe and Herdt, 1992). In addition, the form and level of Se supplementation affects subsequent Se/GPx concentrations. Inorganic Se in the form of selenate (found in both BVP and Vital products used in this study) or selenite is used in many Se supplementation studies. Inorganic Se is used almost exclusively to produce selenoenzymes, whereas organic Se (principally seleno-methionine in a Se-yeast) can be used to produce selenoproteins, and/or may be incorporated non specifically into other proteins in the place of methionine (Weiss, 2005, Ortman, 1999), and as such acts as an endogenous pool of Se (Rock *et al.*, 2001). In the case of inorganic Se (as selenite or selenate), any excess is excreted. Whilst some studies report higher whole blood Se concentrations in cattle fed organic forms of Se (Pavlata *et al.*, 2001; Knowles *et al.*, 1999; Weiss, 2005; Guyot *et al.*,

2007), the relative response to either form of supplementation varies depending on the dose and length of supplementation. In our study, the barium selenate (BVP product) was more successful at raising whole blood Se and GPx concentrations compared with the potassium selenate in combination with dl-alpha-tocopheryl (Vitasel) in animals that remained on the index farm (Se deficient farm) for the duration of the study. This response is most likely due to the higher concentration of Se in the BVP (1 mgSe/kg LBW) compared with the Vitasel (0.067 mgSe/kg LBW) product.

Selenium concentrations approached the threshold of deficiency status (120 µg/kg) by Day 55 in both *Tx1OnFC* and *Tx3Vitasel* animals, both treatment groups that remained on the index farm for the duration of the study (363 days) and both groups were clearly Se deficient between Day 97 and Day 363, however, whilst a similar trend in GPx was noticeable in both groups; neither group reached a deficient GPx status (40 U/ml PCV).

Animal location also affected the Se/GPx status of animals, removing un-supplemented animals (*Tx4OffFC*) from the index farm from Day 41 to Day 166 to an unaffected farm resulted in increased Se and GPx concentrations compared with *Tx1OnFC* and *Tx3Vitasel* animals, which remained on the index farm. Supplementation, however, of animals remaining on the index farm with BVP conferred a similar Se and GPx status when compared with those moved to the unaffected farm, suggesting that BVP was the most suitable product to raise whole blood Se concentrations to an acceptable level for animals remaining on the index farm. Selenium concentrations during the same time period (Day 41 to Day 166), in *Tx1OnFC* and *Tx3Vitasel* animals were predominantly significantly lower compared to *Tx2BVP* and *Tx4OffFC* animals, and were with approaching or below the normal range for Se (120 µg/kg). Studies suggest that at least 100 µg/L Se in whole blood is required to achieve optimal immune capacity and optimal fertility (Pehrson, 1999), therefore, supplementation with BVP should therefore be sufficient to meet the Se requirements of young growing animals on the index farm.

The Se deficient status of the index farm was confirmed when Se and GPx concentrations of *Tx4OffFC* animals declined upon their return to the index farm on Day 166. An increase in the Se and GPx concentrations was observed in *Tx1OnFC*, *Tx3Vitasel* and *Tx4OffFC* animals by Day 280, the mid point of winter housing, despite this rise Se and GPx concentrations were lower compared with *Tx2BVP* animals, and in the case of Se, all three groups remained deficient. It is possible that the increase in Se and GPx was attributable to the winter diet,

animals were housed on Day 181 and received approximately 1 kg of ration per calf per day, this could conceivably improve Se/GPx status.

There are reports of the positive effects of Se supplementation on weight gain in sheep and cattle where the basal diet is deficient in Se, and no effect of Se supplementation where basal diets are marginal or normal in Se content (Pehrson *et al.*, 1999). All animals in the current study gained weight overtime (Figure 1) and there was no difference in mean live weights of three groups of animals that remained on the index farm for the full duration of the study, despite differences in their Se status (Figure 4), indicating that animals were capable of gaining weight despite periods when some groups were considered Se deficient. Therefore the contribution of Se deficiency to the poor performance on the index farm cannot be determined. This is further supported by the fact that supplementation with BVP did not seem to confer any weight or ADG advantage when compared with *Tx1OnFC*, despite their higher Se concentrations, and the below normal Se concentrations in both *Tx1OnFC* and *Tx3Vital* animals (Figure 4).

The biggest differences in live weights and ADG were observed when animals were removed from the index farm from Day 41 to Day 166. Following the initial movement of animals from the index farm, they went through a period of slower growth (Figures 1 and 2). Within a few weeks, however, the weight and ADG of the *Tx4OffFC* animals was almost double that of animals in each of the three other groups, despite the low iodine status of this farm. The initial lag phase in the weight gain of these animals is not unsurprising; most animals will require a period of time to recover from transport and to acclimatise to new management conditions. In Irish studies of Friesian and Friesian cross cattle, similar to the animals in this study, ADGs from calf turn out in the spring to winter housing were between 0.68 and 0.73 kg/day (Keane and Drennan, 2008a,b) compared with only 0.50 ± 0.06 kg/day (*Tx1OnFC*), 0.55 ± 0.04 kg/day (*Tx2BVP*) and 0.47 ± 0.05 kg/day (*Tx3Vital*) for animals in our study that remained on the index farm for the grazing season (Day 27 to Day 181). For the same period, an ADG of 0.67 ± 0.06 kg/day was observed in *Tx4OffFC* animals who were moved to an unaffected farm from Day 41 to Day 166, and this ADG was comparable to those previously reported for typical Irish grazing systems (Keane, 2003; Keane and Drennan, 2008a; Keane and Drennan, 2008b). However, upon the return of the *Tx4OffFC* animals to the index farm (Day 166), their ADGs decreased significantly. Average daily gains improved and were favourable, however, in all four groups between Day 280 and Day 363, when all animals were housed on the index farm [0.77 ± 0.10 kg/day (*Tx1OnFC*), 0.73 ± 0.04 kg/day *Tx2BVP*, 0.66 ± 0.07 kg/day

Tx2Vitasel and 0.69 ± 0.06 kg/day *Tx4OffFC*], and despite the Se deficient status of *Tx1OnFC*, *Tx2Vitasel* and *Tx4OffFC* animals.

Overall mean ADG for all groups over the entire period of the study (363 days) were 0.55 ± 0.05 kg/day (*Tx1OnFC*), 0.55 ± 0.03 kg/day *Tx2BVP*, 0.50 ± 0.06 kg/day *Tx2Vitasel* and 0.55 ± 0.05 kg/day *Tx4OffFC*, and are marginally lower than those reported in Friesian animals over a 310 day period in Grange, Co. Meath (1st year turnout to 2nd year turnout period) of 0.62 to 0.65 kg/day (Keane and O’Riordan, 1988). In a previous investigation (DAFF, 2006), examining animal performance on the index farm, poor growth rates were reported in 10 cattle brought onto and housed on the index farm over a winter period (112 days) These cattle had never been in the vicinity of the index farm before their arrival, and had a mean LW of 345 kgs at the start of the housing period. At the end of 112 days they weighed only 370 kgs, giving an ADG of only 0.22 kg/day, well below normal growth rates for cattle .

Plasma inorganic iodine (PII) concentrations in all animals (measured once only, when *Tx4OffFC* animals returned to the index farm) was either marginal or, in the case of those that moved to the unaffected farm, deficient (Figure 6). In Irish cattle, I is one of the most common mineral deficiencies (Rogers, 2001), with 69 % of abattoir animals (n = 2595) identified as having low PII. Iodine is a component of thyroid hormones thyroxine (T₄) and triiodothyronine (T₃), which are necessary for normal growth and development (Zagrodzki *et al.*, 1998) and is a component of selenoenzymes, as part of the iodothyronine deiodinases which are necessary for the conversion of the biologically inactive T₄ to the active metabolite T₃ via the 5’- monodeiodination of T₄. Additionally, Cu concentrations (Figure 3; measured from Day 41 to Day 363) were below the normal range for all the groups studied. In Ireland, approximately 9 % of abattoir animals (n = 2595) have low blood Cu concentrations, and liver Cu deficiencies are more pronounced in finishing and suckler animals compared with dairy cattle, presumably because mineral supplementation is more routine in dairy cows (Rogers, 2001). Copper concentrations vary with season, with more deficiencies identified in autumn compared with spring.

Two patterns of Cd excess were observed in these animals during the study period: a large Cd peak in July and August 2007, and background Cd exposure. These results gave rise to further investigations, a detailed review of the scientific literature (in Appendix 7, accompanying the *Overview*) and consultations with international experts. A detailed discussion of these results is presented in the *Overview*, and is not considered further here.

Several issues related to animal health were reported during the study. A clinical case of pneumonia was reported in one animal on the index farm on Day 153, at the same time as a drop in ADG. The specific aetiological agent was not determined, and antibiotic treatment was prescribed against secondary bacterial infection, which commonly occurs in cases such as this. In-contact cohort animals were subsequently vaccinated to generate immunological protection against two of the more common viral respiratory agents of cattle (respiratory syncytial virus, RSV; infectious bovine rhinotracheitis, IBR) in Ireland. Respiratory cases due to these agents are not uncommon on farms in Ireland. In a recent study of Irish beef herds submitting bulls for entry to a performance testing station; 73.2% of herds were infected with IBR (O'Grady et al., 2008). It is unlikely that the measured ADG reduction between Day 153 and Day 167 (Figure 2) was other than coincidental, given that it was also observed in the off-farm controls. These latter animals had been removed from the index farm between Day 41 to Day 166 (Tx4OffFC). At the end of this study, elective post mortem examinations were conducted on two animals, including calf A which was considered representative of animals in the study, and calf B which was not. Although these calves were of similar age (7.8 months), calf B (85 kg) weighed less than half that of calf A (195 kg). With calf B, severe bronchopneumonia was undoubtedly a primary contributor to stunting and associated growth retardation lines. In contrast, pathological findings in calf A were limited to ostertagiosis and a light liver fluke infection. Villous atrophy in the intestines was also identified in both animals (Figure 8), for reasons that remain uncertain. Scouring was not reported in any animals during this study. Radiodense growth retardation lines were also identified in the long bones of five of eight animals from the study submitted for radiology examination in April 2008.

Conclusions

Selenium supplementation had a positive impact on blood Se status, but was not sufficient to overcome the shortfall in animal performance, suggesting that Se deficiency may be just a component of a more complex interaction. In support of this view, marginal to deficient concentrations of Cu and iodine (I) were also observed in all groups. In *Study 3*, the results of a detailed post mortem of a single representative study animal were unexceptional. The presence of radiodense growth retardation lines in a number of animals examined is consistent with poor calf growth, but does not provide any definitive insight into the cause of the problem. Two patterns of Cd excess were observed during this study: a large Cd peak and background Cd exposure. The significance of these findings is uncertain but subject to further

study. Understanding the potential complex interaction between element excesses and deficiencies on the index farm could prove important.

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