# An investigation into the effects of maternal supplementation with excess iodine on the mechanisms and impacts of reduced IgG absorption in the lamb postpartum

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## Abstract

An experiment was conducted to determine: (1) the effect of excess maternal I supplementation on the thyroid hormone status of the ewe and her progeny; (2) potential mechanisms underpinning the failure of passive transfer associated with excess I and (3) the growing lambs' response to natural gastrointestinal infection. Twin-bearing ewes received one of two treatments ($n$ 32/treatment group): basal diet (C) or C plus 26·6 mg of iodine/ewe per d (I), supplied as calcium iodate. Ewes were individually fed from day 119 of gestation to parturition. Progeny of I ewes had lower ($P < 0·01$) serum IgG concentrations from 24 h to 28 d postpartum but higher serum IgG concentrations at day 70 postpartum ($P < 0·05$). I supplementation increased the relative expression of Fc receptor, IgA, IgM high affinity and polymeric Ig receptor in the ileum of the lamb at 24 h postpartum; however, thyroid hormone receptor-$\beta$ (*THRB*) and $\beta$-2-microglobulin (*B2M*) expression declined ($P < 0·05$). Progeny of I ewes had higher growth rates to weaning ($P < 0·05$) and lower faecal egg count (FEC) for *Nematodirus battus* ($P < 0·05$) between weeks 6 and 10 postpartum. In conclusion, excess maternal I supplementation negatively affected the thyroid hormone status, serum IgG concentration, ileal morphology and the gene expression of *THRB* and *B2M* in the ileum and ras-related protein (RAB) RAB25 and the mucin gene (MUC) *MUC1* in the duodenum of the lamb postpartum. These effects were followed by an enhancement of average daily gain and lower *N. battus* FEC in the pre-weaning period of I-supplemented lambs.

**Key words: Ewes: Nutrition: Pregnancy: Colostrum IgG: Iodine**

Estimates of ovine pre-weaning mortality currently range from 10 to 30 % wordwide, with the neonatal period being the most vulnerable in this regard[1]. Hypothermia, starvation and infectious disease have been identified as the major causes of mortality within the first 3 d of life[2]. With regard to infection, gastrointestinal nematodes (GIN) have been recognised as the most pervasive problem facing sheep production enterprises[3]. Of these, *Nematodirus battus* has been identified as one of the most prevalent parasites affecting animals in cool, temperate climates; with lambs susceptible to infection from 5 weeks of age[4]. Subclinical infection with GIN results in significant production losses due to weight loss and reduced weight gain, particularly in young, naïve animals[3]. The hypo-immuno-competent nature of the newborn ruminant increases its reliance on acquiring passive immunity in early life[5,6]. Therefore, adequate colostrum consumption is critical to provide nutrition and promote Ig transfer; thus augmenting the ensuing health and survival of the neonatal lamb[7]. Ig are plasma proteins produced by lymphocytes in the mammalian bloodstream in response to foreign antigens. They are part of the innate immune system, which is the primary line of defense against pathogens and therefore are essential in the development of disease resistance[8]. Previous work has found that young lambs are capable of mounting a specific antibody response to *N. battus*, which has been associated with the majority of adult worms being rejected by 21 d post infection[9]. As IgG constitutes approximately 92 % of Ig found in ovine colostrum[10], it the most prevalent Ig class and provides adequate immunity to the neonatal lamb during the initial weeks of independent life[11].

Previous work has outlined that excess I supplementation to ewes in late gestation alters the thyroid hormone status of the newborn lamb, which results in a failure to acquire passive immunity, evidenced by a reduced serum IgG concentration at 24 h postpartum[6,12]. This has been shown to occur independently of colostrum intake and colostrum IgG concentration[13] thus suggesting that there is an alteration in the functioning of

the lambs' intestinal absorptive process which occurs *in utero*. The primary site of IgG absorption in the newborn is the lower ileum[14]; with a previous experiment identifying a link between excess maternal I supplementation and the failure of passive transfer (FPT) of IgG[13]. These authors concluded that FPT was mediated through the thyroid hormone status of the animal and influenced the expression of Ig-related genes in the intestinal tract immediately postpartum[12].

Therefore, the objectives of this study were as follows: (1) to determine the effect of excess I supplementation to the pregnant ewe on the thyroid hormone status of both the ewe and her progeny at parturition; (2) to identify potential mechanisms responsible for FPT by examining the expression profiles of selected genes in the ileum, duodenum, thyroid and perirenal adipose tissue of the lamb at 24 h postpartum; and (3) to assess the effect of FPT on the growing lambs' response to gastro-intestinal infection.

## Methods

All procedures involving ewes and lambs in this study were conducted under experimental licence from the Irish Medicines Board in accordance with the European Union (protection of animals used for scientific purposes) regulations 2012 (S.I. No. 543 of 2012). This study was conducted at University College Dublin, Lyons Research Farm, Newcastle, Co. Dublin.

### Pre-experimental animal management

Ewes were confirmed as pregnant and twin-bearing to a synchronised oestrus (intervaginal progestagen pessaries; Chronogest and Folligon, Intervet Ireland Ltd) followed by an intramuscular injection of 500 IU (2·5 ml) pregnant mare serum gonadotropin (Chronogest and Folligon, Intervet Ireland Ltd), following a transabdominal ultrasound on day 77 post insemination (inseminated with fresh diluted semen, at a rate of 20 million spermatozoa per uterine horn, using laparoscopic artificial insemination (AI)). Before selection, the ewes were blocked on the basis of live weight (75·5 (SE 2·29) kg) and balanced for body condition score (BCS) (2·96 (SE 0·06)), age (3·4 (SE 0·95) years), ewe breed and lamb-sire breed. BCS assessments were made by a trained technician and ewes were scored on a scale of 0–5[15].

### Nutritional treatments

On day 113 of gestation, ewes were allocated to one of two nutritional treatment groups and transferred to individual, wooden-slatted pens measuring 1·1 × 1·4 m. The animals were allowed a 7-d adaption period before experimental feeding began on day 119 of gestation. All ewes were fed to meet 100 % of predicted metabolisable energy (ME) requirements for mature, gestating twin-bearing ewes[16,17]. Ewes received either a basal diet (C), which included mineral and I supplementation or they received C plus an additional daily allowance of 26·6 mg of I supplied in the form of calcium iodate (I, 42·9 mg/ewe per day; Devenish Nutrition). I inclusion levels were not confirmed analytically and are based on calculated formulation. This level of I equates to the level offered to ewes in a recently reported study[6] and is reflective of the level of intake achieved when ewes are offered free access mineral supplements indoors[18].

### Feeding

ME requirements, based on those outlined by the Agricultural and Food Research Council Technical Committee[16] and including amendments[17], were calculated individually for each ewe[19]. Silage was offered at 08.00 hours each morning following the removal of the previous days' silage refusals. The quantity of concentrate offered was calculated for each individual animal depending on the level of ME received from the silage and their individual ME allocation as per treatment group. Concentrates were offered daily at 09.00 hours or twice daily at 09.00 and 17.00 hours in equal allocations when daily allocation exceeded 500 g (fresh weight)/ewe. The iodine supplement was manually mixed into the concentrate on the day before feeding. Before mixing, the iodine allowance was first added to 20 g (fresh weight) of the concentrate that was used as a carrier for the supplement. Before the mineral inclusion, this carrier was dried using forced air circulation at 55°C for 72 h, following which it was ground through a 0·8 mm screen using a Christy and Norris hammer mill (Christy Turner Ltd). The daily concentrate allowance was then adjusted to take into account the carrier contribution. Ewes received an 18 % crude protein (CP) concentrate which comprised barley (40 %), soya hulls (29 %), whole-wheat distillers (25 %) and molasses (4 %), on a DM basis. In addition, the concentrate offered contained minerals (2 %) and cane molasses (2 %). The level of I in C was in excess of standard dietary requirements because of the inclusion of I in the mineral portion of C. The chemical composition of the feedstuff offered is outlined in Table 1. Representative samples of both silage and concentrate were taken each morning and frozen at –20°C for subsequent proximate analysis. Ewes had continuous access to clean, fresh drinking water at all times. Post lambing, all ewes were reverted to C and received grass silage *ad libitum* plus concentrates (1 kg), fed twice daily in equal allocations. At 3 d postpartum, all ewes were turned out onto a perennial rye-grass pasture and were rotationally grazed as a single group along

**Table 1.** Chemical composition of silage and concentrate offered to ewes during late gestation

| Compositions (g/kg) | Silage | 18 % concentrate* |
|---|---|---|
| DM | 232·8 | 759·4 |
| Crude protein | 140·4 | 176·8 |
| Neutral detergent fibre | 428·8 | 274·6 |
| Acid detergent fibre | 269·5 | 154·6 |
| Acid detergent lignin | 19·0 | – |
| Starch | – | 249·7 |
| Diethyl ether extract | 41·0 | 28·8 |
| Ash | 83·7 | 46·9 |
| Gross energy (MJ/kg) | 16·12 | 17·1 |
| Metabolisable energy (MJ/kg) | 10·0 | 12·03 |

* Percentage of crude protein in the concentrate; 18 % crude protein.

with their lambs. At this stage, concentrate supplementation was discontinued.

### Ewe measurements and lambing data

Ewes were assessed for BCS and weighed on an electronic scale (Prattley), with live weights recorded electronically (Tru-Test Group) on day 114 of gestation, 24 h, day 40 and 98 postpartum. A 10 ml blood sample was collected from all ewes on day 119 of gestation (trial start date) and 24 h postpartum via jugular venepuncture using heparinised vacutaiers (ref: 367 526; Becton, Dickinson and Company). Blood samples were immediately placed on ice and centrifuged at 4°C and 1800 $g$ for 15 min after which the plasma was pipetted into pour-off tubes and frozen at –20°C until further analysis.

All ewes lambed down in their individual pens where they remained with their lambs until 72 h postpartum. Within the 1st hour after parturition, the navel of each lamb was dipped in a 10 % I (Ritchey) solution, to aid in the control of erysipelas polyarthritis (joint-ill), following which time of birth, birth weight, sex and the level of assistance required by the ewe at lambing (referred to hereafter as lambing difficulty; LD) were recorded for each lamb. LD was assessed, by trained observers, using a score between 1 and 4 where 1 referred to a natural unassisted lambing and 4 referred to a caesarean section. The time of birth of the first lamb was used to calculate the gestation length (GL) of the ewe, with 12.00 hours on the day of AI taken as 0 h. At 24 h postpartum all lambs were ear-tagged with an electronic identification tag. All incidences of erysipelas polyarthritis (joint-ill) and *Escherichia coli* were recorded in lambs from birth until day 14 postpartum. *E. coli* was diagnosed in lambs that presented with a watery mouth, a distended abdomen and showed obvious signs of pain, lack of appetite and isolation from the dam.

### Hand milking

At parturition sixteen ewes per treatment group were randomly selected and an udder cover placed on them, until 24 h postpartum in order to prevent the lambs from suckling. Ewes were hand milked at 1, 10 and 18 h postpartum as previously described[18]. In brief, each ewe received an intramuscular injection of 10 IU of oxytocin (Oxytocin-S; Intervet Ltd) immediately before each milking to ensure total milk let-down[20]. Ewes were milked out completely by hand and the total volume was recorded after each milking. From this, the total volume produced up to 18 h postpartum was calculated. For the determination of total solid (TS) and CP content, a colostrum sample (25 ml) was taken at the 1 h milking and stored at –20°C until required.

Following each milking, the lambs were fed measured quantities of colostrum from their dam via a stomach tube. Depending on the yield of colostrum, lambs received the maximum amount of colostrum available, within the range of 20–50 ml colostrum per kg birth weight. The quantity of colostrum fed to each lamb was recorded.

### Lamb blood sample collection

At 1 h postpartum, and before stomach tubing where appropriate, a 10 ml plasma blood sample was collected via jugular

venepuncture in heparinised vacutaiers (BD; Ref: 367 880) from all lambs. The blood samples were immediately placed on ice and centrifuged at 4°C at 1800 $g$ for 15 min, after which, the plasma was pipetted into pour-off tubes and stored at –20°C until further analysis. At 24 h postpartum (and before euthanasia where appropriate) and fortnightly thereafter until day 70, a blood sample was collected via jugular venepuncture in non-heparinised vacutaiers (BD; Ref: 368 975) from all lambs. Blood samples were stored at room temperature for 1 h before storage at 4°C where they remained for 24 h. Samples were subsequently centrifuged at 4°C and 1800 $g$ for 15 min to obtain the serum, which was then frozen at –20°C until further analysis.

### Organ collection and small intestinal morphology

At 24 h postpartum, a subgroup of twenty-four lambs ($n$ 12/treatment group) were euthanised with Euthatal (pentobarbitone sodium BP; Merial Animal Health Limited) at a rate of 1 ml/kg body weight. This resulted in eighteen ewes per treatment rearing twin lambs and twelve ewes per treatment rearing single lambs (defined hereafter as rearing rank: twin = 2 and single = 1). Before euthanasia, the rectal temperature of each animal was recorded. Following euthanasia the liver, spleen, kidneys, perirenal adipose tissue, heart, lungs, thyroid and digestive tract were dissected and weighed. After excision, the abomasum was emptied along the mesentery and the contents collected in a 50 ml sterile container (Sarstedt Ltd). The pH of the abomasal contents was then assessed using an Orion 3-Star pH meter (Thermo Fisher Scientific Inc.).

Intestinal tissue from the duodenum (approximately 10 cm from the abomasum) and ileum (approximately 10 cm from the ileo-caecal junction) was aseptically isolated and fixed in 10 % phosphate buffered formalin for villus height (VH) and crypt depth (CD) measurements. Additional sections of the duodenum and ileum were excised, emptied by dissecting along the mesentery and rinsed using PBS (Oxoid; Thermo Fisher Scientific Inc.). Approximately 1–2 g of duodenum, ileum, thyroid and perirenal adipose tissue were collected and placed in 10 ml tubes (Sarstedt Ltd) containing 5 ml of RNAlater™ (Applied Biosystems Ltd), where they were stored for 24 h. The RNAlater™ was then removed and the tissue samples were stored at –80°C.

### Lamb weight and factory data collection

All lambs were weighed fortnightly from days 14–70 postpartum and once every 28 d thereafter until slaughter. Lambs were weighed on an electronic scale and live weight recorded electronically. Lambs were slaughtered once they reached 45 kg live weight. Any lambs drafted before or after this weight was reached had their days-to-slaughter corrected for live weight before statistical analysis. At slaughter, carcass weights were recorded and subsequently used to calculate kill-out percentage (KO %).

### Laboratory analysis

*Proximate analysis*. The DM content (g/kg) of the concentrate and grass silage was determined by drying the samples at 55°C

**Table 2.** Major and trace element composition of silage and concentrate offered to ewes during late gestation*

|  | Silage | Concentrate |
|---|---|---|
| Major elements (g/kg DM) |  |  |
| Ca | 4·9 | 4·48 |
| Na | 1·6 | 2·78 |
| Mg | 1·4 | 2·08 |
| K | 31·3 | 10·78 |
| P | 2·7 | 4·40 |
| Cl | 11·5 | 4·58 |
| Trace elements (mg/kg DM) |  |  |
| Cu | 9·3 | 8·8 |
| Fe | 608·0 | 237·0 |
| Mn | 71·0 | 71·3 |
| Zn | 36·0 | 94·0 |
| I | 1·5 | 3·9 |
| Co | 0·2 | 1·3 |
| Mo | 4·3 | 1·6 |
| Se | 0·2 | 0·6 |

* Mineral analysis of silage and concentrate, without the addition of the I supplement, was carried out by Sciantec Analytical Services Ltd.

for 72 h in a ventilated oven with forced-air circulation. N content (g/kg DM) of the silage and concentrate was determined using a LECO FP528 instrument (Leco Instruments Ltd) according to the method of Dumas[21]. The N concentration of the concentrate and grass silage was multiplied by 6·25 to determine CP concentrations[22]. The neutral detergent fibre (assayed with and without a heat stable amylase for concentrate and grass silage samples, respectively, and without sodium sulphite for both feeds; expressed inclusive of residual ash), acid detergent fibre (expressed inclusive of residual ash) and acid detergent lignin (expressed inclusive of residual ash) concentrations (g/kg DM) of the feedstuff were determined[23] using an ANKOM[200] Fibre Analyzer (ANKOM Technology). Ash concentrations (g/kg DM) of the feedstuff were determined by complete combustion in a muffle furnace at 550°C for 4 h. The gross energy concentration (MJ/kg DM) of the silage and concentrate was determined using bomb calorimetry (Parr 1281 bomb calorimeter; Parr Instrument Company)[24], whereas the ME concentration (MJ/kg DM) was calculated using the following equation[24]:

$$100 \times ME/DE = 86\cdot38 - 0\cdot099\, CFo - 0\cdot196\, CPo,$$

where CFo is crude fibre in the organic matter and CPo is CP in the organic matter.

Diethyl ether extract concentrations (g/kg DM) of the feedstuff were determined using light petroleum ether and Soxtec instrumentation (Tecator). The concentration of starch in the concentrate was determined using the Megazyme Total Starch Assay Procedure[21]. Mineral analysis of silage and concentrate without the addition of the I supplement was carried out by Sciantec Analytical Services Ltd and is presented in Table 2.

*Blood sample analysis.* Total serum Ig concentration (g/l) was determined using the zinc sulphate turbidity test[25]. These results were then reduced by a factor of 0·09 to provide the IgG-only content of the serum[26]. The efficiency of IgG absorption within the first 24 h postpartum was further calculated. It was assumed that in lambs, as in calves, 0·075 of birth weight is equivalent to blood plasma volume[27]. Therefore, the following equation was used to calculate the IgG absorption efficiency:

$$IgG\ absorption =$$

$$\left( \frac{Birth\ weight \times 0\cdot075 \times Serum\ IgG\ concentration}{Total\ IgG\ fed} \right) \times 100.$$

Plasma total tri-iodothyronine ($T_3$) and total thyroxine ($T_4$) concentrations were determined using commercial RIA kits ($T_3$: KIP1631 or $T_4$: KIP1641; DIAsource ImmunoAssays S.A.) where all samples were measured on the same assay in duplicate. The intra-assay CV were 3·85 and 4·96 % for the $T_3$ and $T_4$ assays, respectively.

*Colostrum analysis.* The N content (g/kg DM) of the colostrum was determined using a LECO FP528 instrument according to the method of Dumas[21]. The N concentration in the colostrum was multiplied by 6·38 to determine CP concentrations[22]. The TS (g/l) content of the colostrum was determined using a direct forced-air method where 3 ml of colostrum and/or milk were placed in an oven for 4 h at 100°C[23].

The colostrum samples were thawed at room temperature the day before laboratory analysis. IgG concentration of the samples was determined by the ELISA method (Ovine IgG ELISA Kit, Cat. No. 7620; Alpha Diagnostic International). Samples were diluted 1:1 000 000 before being assayed in duplicate, with an inter-assay CV of 5 %. The concentration of IgG in the samples was calculated from a standard reference curve containing known concentrations of IgG. Any sample that resulted in an IgG concentration that fell outside the range of the standard reference curve was retested after further dilution according to the test recommendations.

*Duodenal and ileal morphology.* The preserved intestinal segments were prepared using standard paraffin-embedding techniques. Cross-sections of 5 μm thickness of each sample were stained with haematoxylin–eosin[28]. VH and CD were measured on the stained sections of the ileal tissue and only VH measurements were taken from the duodenal tissue, using a light microscope (100× objective) fitted with an image analyser (Image-Pro Plus 9.1; Media Cybernetics). Measurements of twenty well-orientated intact villi and crypts were measured for each animal. VH was measured from the crypt–villus junction to the tip of the villus and CD was measured from the crypt–villus junction to the base.

*Extraction and assessment of RNA integrity.* Total RNA was isolated from the ileum, duodenum, thyroid and perirenal adipose tissues using TRI reagent (Molecular Research Center) according to the manufacturer's instructions, followed by further purification with the GenElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich). In brief, approximately 25 mg of tissue was homogenised in 1·0 ml of Trizol using a steel bead (Qiagen Ltd) and the Qiagen TissueLyser II (2 × 120 s maximum speed). Chloroform (200 μl) was then added to each sample, and following centrifugation (12 000 $g$, 15 min), the aqueous phase was transferred directly onto an RNeasy column (Qiagen Ltd). The RNA was further purified as

per the manufacturer's instructions and included an on-column DNase step (Sigma-Aldrich).

The total RNA was quantified using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific Inc.) and the purity was assessed by determining the ratio of the absorbance at 260 and 280 nm. All total RNA samples with acceptable 260:280 nm ratios were used for further analysis. Total RNA was analysed in an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.) using RNA 6000 Nano LabChips (Caliper Technologies Corp.). The RNA Integrity Number for the ileum, duodenum, thyroid and perirenal adipose tissues were 9·1 (SEM 0·06); 9·0 (SEM 0·08); 9·2 (SEM 0·15) and 8·7 (SEM 0·19), respectively.

### Complementary DNA synthesis and quantitative real-time PCR.

Total RNA from each sample (1 µg) was reverse transcribed using the high capacity complementary DNA (cDNA) reverse transcription kit (Applied Biosystems Ltd) according to the manufacturer's instructions. The purified cDNA was diluted in RNase- and DNase-free water to a volume of 250 µl and stored at –20°C. Oligonucleotide primers were designed with Primer Express™ Software, version 2.0 (Applied Biosystems) and synthesised by MWG-Biotech. All assays were assessed for specificity using dissociation analysis. Assays with efficiencies between 90 and 110 % were deemed acceptable. Target genes quantified in the ileum and duodenum were: Fc fragment of the IgG receptor transporter, α (*FCGRT*), Fc receptor, IgA, IgM high affinity (*FCAMR*), polymeric Ig receptor (*PIGR*), β-2-microglobulin (*B2M*), cellular myelocytomatosis oncogene, *TNFα*, upstream stimulator factor 1 (*USF1*), upstream stimulator factor 2 (*USF2*), thyroid hormone receptor-α (*THRA*), thyroid hormone receptor-β (*THRB*), the ras-related proteins (RAB) *RAB11a* and *RAB25* and the mucin genes (MUC) *MUC1* and *MUC3A*; whereas *THRA*, *THRB* and uncoupling protein 1 (*UCP1*) were quantified in the thyroid and perirenal adipose tissues of lambs. All primer sequences for the target and reference genes were as presented in a previous study[12].

The quantitative PCR (qPCR) assays were performed in a total volume of 20 µl, containing 2 × Fast SYBR PCR Master Mix (Life Technologies Ltd), 1 µl of a forward and reverse primer mix (300 nm each) and 5 µl cDNA. qPCR was carried out in duplicate, on the 7500 ABI Prism Sequence Detection System (Applied Biosystems Ltd). Thermocycling conditions were as follows: 95°C for 10 min, followed by 95°C for 15 s, and 60°C for 1 min for forty cycles.

A total of six potential reference genes: β-actin, glyceraldehydes-3-phosphate dehydrogenase (*GAPDH*), glucose-6-phosphate dehydrogenase, hypoxanthine phosphoribosyltransferase 1 (*HPRT1*), ribosomal protein L19 and succinate dehydrogenase (*SUCD*) were analysed using the geNorm algorithm on the qbase+ package (Biogazelle)[25]. *GAPDH* and *SUCD* were the most stably expressed in the ileum, duodenum and thyroid, and were subsequently used to normalise the gene expression data in these tissues; while *HPRT1* and *SUCD* were used to normalise the gene expression data in the perirenal adipose tissue. The mean $C_t$ values of duplicates of each sample were used by the qBASE plus algorithm to generate the normalised relative quantities for each gene.

### Determination of faecal egg count.

Faecal samples were collected per rectum from all lambs once fortnightly from days 42–70 postpartum. All samples were placed on ice until their return to the laboratory where they were stored at 4°C before assessment. Faecal egg count (FEC) were determined using the modified McMaster method[29,30]. FEC was distinguished separately as '*N. battus*' ($FEC_N$) and 'other trichostrongyles' ($FEC_{OT}$) with the number of eggs/g (epg) enumerated. Before statistical analysis, FEC values were transformed using the natural logarithm ($\log_e(X+25)$) to stabilise the variance.

## Statistical analysis

In this study there are two treatment groups; C and I. The difference ($\mu1 – \mu2$) is expressed as a percentage of $\mu1$: that is, ($\mu1 – \mu2)/\mu1 \times 100$ and denoted by $d$. The CV is by definition $\sigma/\mu1 \times 100$. According to the University College Dublin Animal Research Ethics Committee guidelines the formula is written as $n\ 2 \times (CV/d)^2 \times (1·96 + 0·842)^2$, that is, $n\ 15·7 \times (CV/d)^2$. Lamb performance in terms of growth rate is the most important outcome variable in this study. Comparisons with the control were based on a significance level of 5 % and a power of 80 %. From previous work CV = 18 % and $d$ = 10 %[19]. Taking this into account the sample size per group is $n\ 15·7 \times (13·5/10)^2 = 50·9$. In each treatment, twelve lambs were euthanised, and a natural mortality of 4 % was assumed. Therefore, in order to retain fifty-one lambs per treatment for growth rate studies, thirty-two twin-bearing ewes per treatment were required.

Data were analysed as a completely randomised block design using the mixed model procedure (PROC MIXED) in SAS[31]. The binary data, namely lamb health, was analysed using PROC GENMOD. The individual ewe was considered the experimental unit for all parameters analysed. Descriptive statistics were performed to identify continuous and categorical variables and their distributions analysed to fit the assumptions of normality using the UNIVARIATE procedure. The statistical model used for all parameters included the fixed effect of treatment, whereas the ewe data was adjusted for live weight at the beginning of the experiment by covariance. Date of birth was fitted as a covariate for all lamb parameters analysed. A repeated measure analysis was performed on ewe DM intake, live weight, BCS and colostrum production data, and lamb live weight and FEC data. In all, six covariance structures were compared: compound symmetry, heterogeneous compound symmetry, autoregressive order 1, heterogeneous autoregressive order 1, toeplitz and heterogeneous toeplitz; whereas the covariance structure that yielded the lowest Bayesian information criterion was used in the model. The fixed effects of time (as the repeated constant), treatment, sex, rearing rank, the two-way interaction of ewe nutritional treatment × time and rearing rank × time were included in the model. A total of four ewes (*n* 2/treatment) gave birth to one lamb plus one mummified fetus and these were excluded from the final analysis. Therefore, the final number of ewes used for analysis in each treatment (*n*) were thirty and thirty for the C and I treatments, respectively. There was no interaction between ewe nutritional treatment × rearing rank (P > 0·25); therefore, it was removed

from the model before the final analysis of lamb weight and growth rate data. Rearing rank wasn't a significant factor influencing $FEC_N$ or $FEC_{OT}$ ($P > 0.05$) hence it was removed from the final model before analysis. Lamb birth weight was included as a covariate in the analysis of lamb growth rate data. A Tukey's adjustment was applied where multiple observations were compared in the final analysis. All data presented in the tables and figures are expressed as least-square means with their standard errors. The probability value, which denotes statistical significance, was $P < 0.05$, while values tending towards significance were $0.05 < P < 0.10$.

## Results

### Ewe DM, crude protein and metabolisable energy intake, live weight and body condition score

Dietary treatment had no effect on the DM intake (1·29 *v.* 1·29 (SEM 0·01) kg/d), CP (190 *v.* 189 (SEM 2·72) g/d) or the ME intake (13·26 *v.* 13·20 (SEM 0·13) ME/ewe per d) of ewes throughout the feeding period ($P > 0.05$). I supplementation had no effect on the live weight, BCS or BCS change of the ewes ($P > 0.05$) when assessed throughout the experimental period (data not shown).

### Lambing parameters

I supplementation had no effect on ewe GL, LD or the combined litter weight of lambs at birth, with means ranging from 9·55 to 9·66 (SEM 0·126) kg, for the C and I progeny, respectively ($P > 0.05$). I supplementation did not affect the incidence of joint-ill or *E.coli* infection from birth until day 14 postpartum (P 0·235; data not shown).

### Colostrum yield, IgG concentration and intake

Treatment had no effect on colostrum yield or IgG concentration at 1, 10 and 18 h postpartum or on the total yield to 18 h ($P > 0.05$). There was no effect of I supplementation on the TS or CP concentrations in the colostrum ($P > 0.05$; data not shown).

Dietary treatment had no effect on the intake of colostrum or IgG when measured at 1, 10 and 18 h postpartum or on an absolute basis to 18 h ($P > 0.05$; data not shown). Similarly, there was no effect of dietary treatment on the volume of colostrum or on the quantity of IgG fed, per kg of lamb birth weight, to lambs in both the C and I treatment groups. However, excess I supplementation of the ewe reduced the absorption efficiency of colostral IgG within the first 18 h postpartum ($P < 0.0001$; data not shown).

### Blood metabolites

*Thyroid hormone concentration*. Supplementation of the ewe with excess I in late gestation tended to increase the concentration of total $T_3$ (P 0·07; Table 3) and increased the concentration of total $T_4$ at 24 h postpartum ($P < 0.05$). There was no effect of dietary treatment on the concentration of total $T_4$ in the lamb serum at either 1 or 24 h postpartum ($P > 0.05$). However, lambs born to I-supplemented ewes had a lower

**Table 3.** The effect of prepartum dam nutritional treatment on ewe (*n* 30/treatment) and lamb (*n* 60/treatment) total tri-iodothyronine ($T_3$) and thyroxine ($T_4$) (nmol/l) concentrations

|  | Treatment | | | |
| --- | --- | --- | --- | --- |
|  | C | I | SEM | *P* |
| Ewes (24 h postpartum) | | | | |
| Total $T_4$ | 71·67 | 119·3 | 14·91 | 0·007 |
| Total $T_3$ | 1·86 | 2·44 | 0·284 | 0·073 |
| Lamb (1 h postpartum) | | | | |
| Total $T_4$ | 72·4 | 73·7 | 12·18 | 0·928 |
| Total $T_3$ | 2·74 | 1·56 | 0·301 | 0·002 |
| Lambs (24 h postpartum) | | | | |
| Total $T_4$ | 87·8 | 83·1 | 4·32 | 0·452 |
| Total $T_3$ | 3·71 | 2·88 | 0·412 | 0·091 |

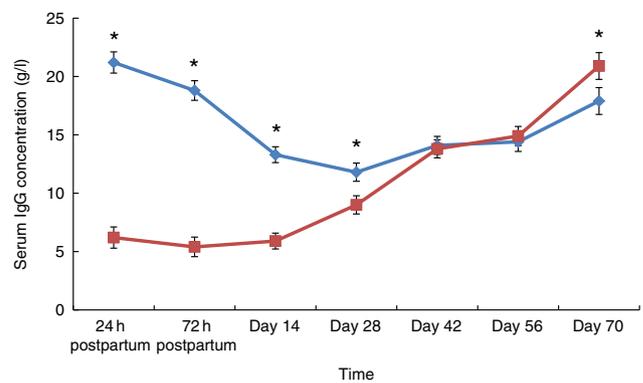C, basal diet, no added supplement; I, ewes supplemented with 26·6 mg of I added as calcium iodate.



**Fig. 1.** The effect of prepartum dam nutritional treatment on the serum IgG concentration of the lamb postpartum. Treatments: C, basal diet, no added supplement (—◆—); I, C plus 26·6 mg of iodine added as calcium iodate (—■—). Values are least-square means (*n* 60 lambs per treatment for 24 h postpartum and *n* 48 lambs per treatment thereafter), with their standard errors. * Significant difference between C and I ($P < 0.01$). For a colour figure, see the online version of the paper.

serum concentration of total $T_3$ at 1 h ($P < 0.05$). This effect tended to remain until 24 h postpartum ($P = 0.09$).

*Lamb serum IgG*. The effect of dietary treatment on the serum IgG concentration of the progeny is presented in Fig. 1. Progeny of I ewes had lower concentrations of serum IgG from 24 h to day 28 postpartum ($P < 0.01$); however, this had reversed on day 70 postpartum ($P < 0.05$) with the progeny of I ewes having higher concentrations of serum IgG when compared with the progeny of C ewes.

### Organ weights and small intestinal morphology

Excess I supplementation to the ewe had no effect on the rectal temperature or abomasal pH of the progeny at 24 h postpartum ($P > 0.05$; data not shown). There was no effect of excess I supplementation to the ewe on the weight of the fetal digestive tract, liver, lungs, thyroid and kidneys when assessed on an absolute basis, at 24 h postpartum ($P > 0.05$). The above results remained non-significant after correction for fetal birth weight
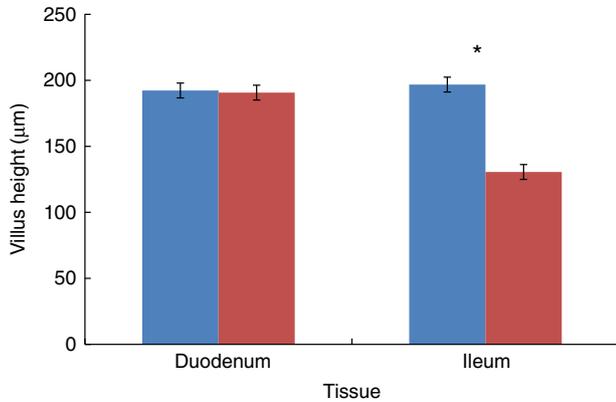
**Fig. 2.** The effect of prepartum dam nutritional treatment on the villus height (μm) in the duodenum and the ileum of the lamb at 24 h postpartum. Treatments: C, basal diet, no added supplement (■); I, C plus 26·6 mg of iodine added as calcium iodate (■). Values are least-square means (n 12 lambs per treatment), with their standard errors represented by vertical bars. * Significant difference between C and I for villus height in the ileum (P < 0·01). For a colour figure, see the online version of the paper.
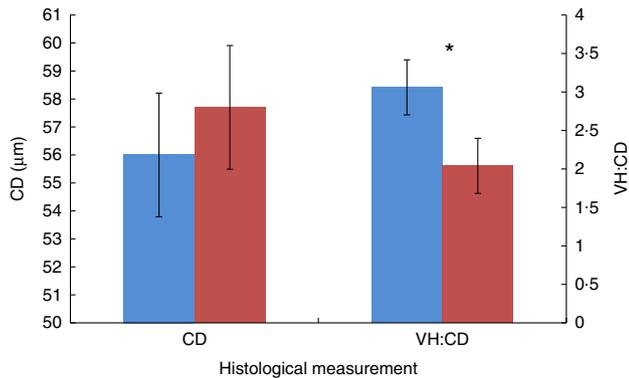


**Fig. 3.** The effect of prepartum dam nutritional treatment on the crypt depth (CD) and the villus height (VH):CD ratio in the ileum of the lamb at 24 h postpartum. Treatments: C, basal diet, no added supplement (■); I, C plus 26·6 mg of iodine added as calcium iodate (■). Values are least-square means (n 12 lambs per treatment), with their standard errors represented by vertical bars. * Significant difference between C and I for VH:CD ratio in the ileum (P < 0·01). For a colour figure, see the online version of the paper.

(data not shown). Dietary treatment affected the weight of the spleen (P = 0·05), which was 19 % lighter, and tended to affect the weight of the perirenal adipose tissue (P = 0·06), which was 12·8 % lighter in the progeny of I ewes; however, these effects were not present after correction for fetal birth weight (P > 0·05). After correction for birth weight, the progeny of C ewes tended to have a lighter heart weight (P = 0·09; data not shown).

The effect of excess I supplementation on VH in the duodenum and ileum, and CD and VH:CD ratio in the ileum of lambs at 24 h postpartum are shown in Fig. 2 and 3. Histological examination of the duodenum indicated there was no effect of dietary treatment on the VH at 24 h postpartum (P > 0·05). Progeny of the I ewes had a reduced VH and VH:CD ratio (P < 0·01), despite the lack of difference in ileum CD (P > 0·05) at 24 h postpartum.

### Gene expression data

Excess I supplementation to the ewe down-regulated the expression of B2M, and THRB in the ileum of the lamb at 24 h postpartum; while the expression of FCAMR and PIGR were up-regulated (P < 0·05; Table 4). Supplementation of the ewe with excess I had no effect on the expression of FCGRT, THRA, USF1, USF2, RAB11a, TNF and MUC3a in the ileum of lambs at 24 h postpartum (P > 0·05). Maternal dietary treatment tended to affect the mRNA expression of albumin (ALB) in the ileum (P = 0·07), with lower levels expressed in the progeny of I-supplemented ewes. In the duodenum, there was no effect of maternal supplementation with excess I on the expression of FCAMR, FCGRT, PIGR, THRA, THRB, USF1, USF2, RAB11a and TNF in lambs at 24 h postpartum (P > 0·05). The progeny of I-supplemented ewes had an increased expression of RAB25, whereas the expression of MUC1 was down-regulated in the duodenum (P < 0·05). There was no effect of dietary treatment on the mRNA expression of THRA and THRB in the thyroid tissue of lambs; however, the expression of THRB was down-regulated in the perirenal adipose tissue of I progeny (P < 0·05; Table 5). In addition, there was no effect of excess I supplementation to the ewe on the expression of UCP1 in the perirenal adipose tissue of lambs in the present study (P > 0·05).

### Lamb performance

*Live weight*. There was no effect of excess I supplementation to the ewe on the live weight of the progeny from birth to day 98 postpartum (weaning; P > 0·05; Fig. 4.). There was an effect of rearing rank (P = 0·001) from day 14 until weaning at day 98 postpartum; where single reared lambs remained heavier than those reared as twins. However, there was no interaction between ewe dietary treatment and rearing rank (P > 0·05) for lamb live weight.

*Average daily gain*. There was no effect of dietary treatment on the average daily gain (ADG) of progeny from days 0 to 42 postpartum (P > 0·05; Table 6). The progeny of the I ewes had higher growth rates from days 42 to 70 postpartum (P = 0·08) and from days 70 to 98 (P < 0·05). Subsequently, the I progeny had increased growth rates (P < 0·05) from days 0 to 98 postpartum (birth to weaning; 291 v. 271 (SEM 9·4) g/d, respectively).

*Slaughter data*. There was no effect of excess I supplementation on the carcass weight of lambs (P = 0·72; data not shown), the ADG from birth to slaughter or weaning to slaughter (P = 0·18; Table 6) and the KO % of lambs at slaughter (P = 0·64).

### Faecal egg counts

The results of FEC_N for lambs are shown in Table 7. There was an overall effect of excess I supplementation, such that I lambs had lower mean FEC_N when compared with the progeny of C ewes (P = 0·01). A treatment × time interaction was present whereby on day 42 I lambs had lower FEC_N than C lambs; however, this wasn't present on days 56 or 70 postpartum.

**Table 4.** The effect of prepartum dam nutritional treatment on the normalised relative quantity of Ig-associated genes; *TNFα* and mucins in the ileum and duodenum of lambs at 24 h postpartum
(*n* 12 lambs per treatment)

| | Tissue type | | | | | | Treatment (*P*) | |
| | Ileum | | | Duodenum | | | | |
| | Treatment | | | | | | | |
| Genes | C | I | SEM[2] | C | I | SEM | Ileum | Duodenum |
|---|---|---|---|---|---|---|---|---|
| *THRA* | 1·57 | 1·47 | 0·217 | 1·35 | 1·27 | 0·147 | 0·633 | 0·541 |
| *THRB* | 2·05 | 1·10 | 0·394 | 1·37 | 1·30 | 0·204 | 0·027 | 0·708 |
| *FCGRT* | 1·04 | 1·19 | 0·146 | 1·04 | 1·00 | 0·060 | 0·466 | 0·691 |
| *FCAMR* | 1·51 | 2·65 | 0·375 | 1·11 | 1·02 | 0·276 | 0·041 | 0·757 |
| *PIGR* | 0·87 | 1·21 | 0·149 | 1·02 | 0·77 | 0·340 | 0·043 | 0·490 |
| *B2M* | 1·10 | 0·83 | 0·083 | 0·92 | 0·89 | 0·046 | 0·023 | 0·641 |
| *ALB* | 1·70 | 0·87 | 0·358 | 1·22 | 0·92 | 0·257 | 0·066 | 0·416 |
| *USF1* | 0·99 | 1·16 | 0·124 | 1·06 | 1·08 | 0·057 | 0·344 | 0·830 |
| *USF2* | 0·92 | 1·03 | 0·099 | 1·10 | 1·09 | 0·102 | 0·434 | 0·903 |
| *RAB11a* | 1·50 | 1·22 | 0·193 | 1·25 | 1·27 | 0·026 | 0·124 | 0·496 |
| *RAB25* | 1·14 | 0·97 | 0·105 | 0·86 | 1·06 | 0·085 | 0·266 | 0·027 |
| *TNF* | 1·27 | 1·55 | 0·206 | 0·91 | 1·01 | 0·095 | 0·346 | 0·498 |
| *MUC1* | 1·07 | 0·97 | 0·134 | 0·93 | 0·52 | 0·186 | 0·619 | 0·028 |
| *MUC3a* | 6·06 | 7·27 | 1·913 | 3·37 | 3·70 | 0·998 | 0·506 | 0·818 |

C, basal diet, no added supplement, I, ewes supplemented with 26·6 mg of iodine added as calcium iodate; *THRA*, thyroid hormone receptor-α; *THRB*, thyroid hormone receptor-β; *FCGRT*, Fc fragment of the IgG receptor transporter, α; *FCAMR*, Fc receptor, IgA, IgM high affinity; *PIGR*, polymeric Ig receptor; *B2M*, β-2-microgulobulin; *ALB*, albumin; *USF1*, upstream stimulator factor 1; *USF2*, upstream stimulator factor 2; *RAB*, ras-related protein; *MUC*, mucin genes.

**Table 5.** The effect of prepartum dam nutritional treatment on the normalised relative quantity of Ig-associated genes in the thyroid and perirenal adipose tissue of lambs at 24 h postpartum
(*n* 12 lambs per treatment)

| | Tissue type | | | | | | Treatment (*P*) | |
| | Thyroid | | | Perirenal adipose tissue | | | | |
| | Treatment | | | | | | | |
| Genes | C | I | SEM | C | I | SEM | Thyroid | Perirenal adipose tissue |
|---|---|---|---|---|---|---|---|---|
| *THRA* | 1·07 | 1·10 | 0·129 | 0·98 | 0·89 | 0·123 | 0·871 | 0·490 |
| *THRB* | 1·12 | 1·08 | 0·140 | 1·21 | 0·87 | 0·091 | 0·872 | 0·029 |
| *UCP1* | 0·85 | 0·77 | 0·288 | 1·30 | 1·36 | 0·125 | 0·852 | 0·608 |

C, basal diet, no added supplement; I, ewes supplemented with 26·6 mg of I added as calcium iodate; *THRA*, thyroid hormone receptor-α; *THRB*, thyroid hormone receptor-β; *UCP1*, uncoupling protein 1.
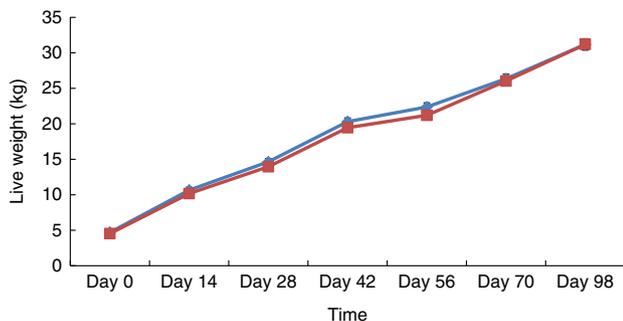


**Fig. 4.** The effect of prepartum dam nutritional treatment on lamb live weight postpartum. Treatments: C, basal diet, no added supplement (——◆——); I, C plus 26·6 mg of iodine added as calcium iodate (——■——). Values are least-square means (*n* 60 lambs per treatment for 'day 0' (birth weight) and *n* 48 lambs per treatment thereafter), with their standard errors. For a colour figure, see the online version of the paper.

There was further evidence of a time effect whereby the number of faecal epg gradually increased with age at sampling.

There was no effect of excess I supplementation on the number of $FEC_{OT}$ observed from lambs (*P* > 0·05; Table 7); however, there tended to be a time effect where the number of $FEC_{OT}$ increased with age (*P* = 0·08).

## Discussion

Maternal I supplementation of the pregnant ewe has been shown to negatively influence serum IgG concentrations in her progeny immediately postpartum[11], however, neonatal lambs are dependent upon passively acquiring maternal antibodies from the colostrum to confer disease resistance in early life. Therefore, the primary objectives of this study were: (1) to determine the effect of excess I supplementation to the pregnant ewe on the thyroid hormone status of both the ewe and her progeny at parturition; (2) to determine potential mechanisms responsible for FPT by examining the expression profiles of selected genes in the ileum, duodenum, thyroid and perirenal adipose tissue of the lamb at 24 h postpartum; and

**Table 6.** The effect of prepartum dam nutritional treatment on lamb average daily gain (ADG), days-to-slaughter and kill-out percentage (n 48/treatment)

| | Treatment | | | |
| | C | I | SEM | P |
|---|---|---|---|---|
| ADG between days (g/d) | | | | |
| Days 0†–14 | 332 | 314 | 13·5 | 0·341 |
| Days 0–42 | 313 | 313 | 9·8 | 0·999 |
| Days 0–98 | 271 | 292 | 9·4 | 0·029 |
| Day 98‡–slaughter* | 156 | 156 | 11·5 | 0·981 |
| Day 0–slaughter | 213 | 226 | 9·3 | 0·178 |
| Slaughter data | | | | |
| Days-to-slaughter | 193 | 187 | 10·3 | 0·557 |
| Kill out percentage | 46·19 | 46·58 | 0·827 | 0·637 |

C, basal diet, no added supplement; I, ewes supplemented with 26·6 mg of I added as calcium iodate.
* Lambs were drafted for slaughter when they reached 45 kg body weight.
† Day 0 = birth.
‡ Day 98 postpartum = weaning.

(3) to assess the effect of FPT on the growing lambs' response to gastrointestinal infection. The results from this study include a down-regulation of *THRB* and *B2M* and a reduction in VH in the ileum of I-supplemented progeny. Supporting previous work[11], which saw a decline in free $T_3$, a decline in total $T_3$ concentrations in the lamb were also observed in this study. These observations may explain in part the decline in IgG absorption at 24 h postpartum. However, despite the evident reduction in IgG absorption in lambs born to I-supplemented ewes, these lambs exhibited lower $FEC_N$ in response to a natural parasitic infection and enhanced growth performance. These observations have led us to hypothesise that lambs born to I-supplemented ewes develop a superior ability to fight infection to *N. battus* in later life.

Most dietary I becomes incorporated as a constituent of the thyroid hormones $T_4$ and $T_3$ in mammalian species[32,33]. In agreement with this, increased concentrations of both the thyroid hormones were observed in ewes supplemented with excess I in the present study. While placental transfer of both $T_3$ and $T_4$ is absent during the final two-thirds of gestation, the production of thyroid hormones by the fetus remains dependent of the uptake of iodide from maternal circulation[34]. However, in the present study, lambs born to I-supplemented ewes had lower concentrations of total $T_3$ at 1 and 24 h postpartum and lower expression of *THRB* in the ileum at 24 h postpartum. This finding is in agreement with previous studies[6,12,35] and may be partially explained by a disruption to the conversion of $T_4$ to the biologically active $T_3$. In comparison with previous work, lower concentrations of total $T_4$ were observed in this study[6]. Neonatal $T_4$ concentrations are dependent on a thyroid-stimulating hormone surge which is further reliant on the intake of milk by the neonate after birth[34]. Therefore, the lack of milk consumption by the newborn lamb before blood sample collection at 1 h postpartum may in part explain the lower concentrations of $T_4$ in the lambs at 1 h postpartum.

The level of I offered to ewes in the present study was in excess of dietary I requirements for both the C and I treatment groups[36–38] but did not exceed the stated toxicity level of 50 mg/ewe per d[39]. Despite this increase in I intake, in excess

**Table 7.** The effect of prepartum dam nutritional treatment on the faecal egg count (FEC) for *Nematodirus battus* ($FEC_N$) and other trichostrongyle ($FEC_{OT}$) in lambs (Least-square means with their standard errors; geometric means and 95 % confidence intervals; n 48/treatment)

| Treatments | Day | $FEC_N$ | | | | $FEC_{OT}$ | | | | $P^*$ | |
| | | Least-square mean† | SEM | Geometric mean | 95 % CI | Least-square mean | SEM | Geometric mean | 95 % CI | $FEC_N$ | $FEC_{OT}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C | Overall | 5·7 | 0·09 | 438 | 368, 510 | 5·3 | 0·10 | 258 | 210, 307 | 0·014 | 0·166 |
| I | Overall | 5·4 | 0·09 | 334 | 275, 340 | 5·0 | 0·19 | 198 | 163, 233 | | |
| C | 42 | 5·2 | 0·16 | 293 | 180, 407 | 5·0 | 0·18 | 183 | 107, 258 | 0·033 | 0·426 |
| I | 42 | 4·7 | 0·17 | 211 | 103, 318 | 4·8 | 0·27 | 133 | 68, 199 | | |
| C | 56 | 5·8 | 0·16 | 415 | 283, 547 | 5·3 | 0·15 | 266 | 168, 363 | 0·216 | 0·549 |
| I | 56 | 5·5 | 0·17 | 313 | 212, 414 | 5·1 | 0·22 | 199 | 135, 261 | | |
| C | 70 | 6·2 | 0·15 | 586 | 470, 701 | 5·5 | 0·14 | 292 | 219, 366 | 0·379 | 0·130 |
| I | 70 | 6·0 | 0·15 | 454 | 367, 542 | 5·1 | 0·21 | 219 | 165, 273 | | |

C, basal diet, no added supplement; I, ewes supplemented with 26·6 mg of I added as calcium iodate.
* Treatment P value for the log scale least-square means.
† Log scale least-square means; $\log_e(X+25)$.

of requirement and the elevated production of the thyroid hormones, there were no effects on the performance parameters of the ewes including colostrum yield, composition and IgG concentration. However, in accordance with previous studies, FPT was evident in lambs at 24 h postpartum[6,13,40–42]. Because of the hypo-immunocompetent nature of the newborn ungulate[6], FPT has been linked to increases in susceptibility to infection and subsequent declines in health[43,44]. However, in the present study there was no decline in the number of lambs treated for either joint-ill or *E.coli* between birth and day 14 postpartum. Previous authors have suggested that the decline in serum IgG concentration is a result of a 'preprogramming' effect due to excess I supplementation and mediated through the thyroid hormones in the developing fetus[10,12]. In this study, the lower concentration of serum IgG, observed among lambs born to I-supplemented ewes, was evident until day 32 postpartum, after which the lamb normally produces its own internal antibodies[45]. This highlights the persistency of the pre-programming effect on serum IgG in the lamb; which had been previously reported at 24 and 72 h postpartum[6]. Supplementation of the ewe with minerals and more specifically I prepartum may lead to increased concentrations of the mineral in both the colostrum and milk of the ewe postpartum; however, previous work has shown that the progeny of I-fed dams had reduced serum IgG concentrations when fed either artificial colostrum[42] or colostrum from control-fed ewes[10].

Previous authors have hypothesised that the resultant FPT, following excess I supplementation of the pregnant ewe, may be due to the IgG being rechannelled back into the intestinal lumen[32,37,46]. The absorption of IgG from the colostrum is mediated via the neonatal Fc Receptor (FcRn)[14], which has been linked to both the transcytosis and recycling of IgG[47,48]. The primary site of IgG absorption in the newborn ruminant is in the lower ileum[14] where there are two primary pathways of IgG absorption. First, IgG is bound to FcRn at the apical surface, forming an endosome, within which it is transcytosed to the basal surface, or alternatively, the IgG is engulfed into intestinal cells via fluid phase endocytosis before binding to FcRn and being transcytosed across intestinal epithelial cells[46,49,50]. Therefore, recycling of IgG back into the intestinal lumen requires transport from the basal to the apical surface[48]. Previous authors have reported the localisation of FcRn, which are known to mediate the recycling of IgG back into the intestinal lumen, at the apical side of duodenal crypt epithelial cells[32,37]. In the present study, there was no difference between the expression of the FcRn heavy chain component, *FCGRT*, in the ileum and the duodenum; however, an up-regulation of *RAB25* gene expression was observed in the duodenum. *RAB25* is a member of the Rab GTPases family which are small intracellular proteins associated with the regulation of recycling or transcytosis of the endosome[46]. An *in vitro* study found that suppression of *RAB25* inhibited the bidirectional transcytosis of IgG by 25–50 %[50]. If the opposite effect were true, the up-regulation of *RAB25* in the duodenal tissue examined in the present study may indicate an increase in IgG recycling from the basal to the apical surface of the intestinal tract.

A secondary function of the FcRn receptor is to protect IgG and albumin from catabolism, thus, indirectly increasing the serum half-life of both[51]. Higher serum concentrations of IgG can therefore be limited by the availability of FcRn, as unbound IgG is delivered to lysosomes where it is degraded[37]. Maternal supplementation with excess I down-regulated *B2M* and tended to down-regulate *ALB* in the ileum of lambs in the present study. In mice, a deletion in the light chain component of the FcRn receptor, B2M, has previously been linked to loss of FcRn expression and depletion of maternal IgG transport[52] resulting in lower levels of both serum IgG and albumin[53]. Hence, the down-regulation of *B2M*, in conjunction with the decline in serum IgG concentrations, could indicate reduced expression of functional FcRn and therefore increased levels of IgG catabolism in the intestine of the lamb. A previous study which looked at the expression of *B2M* in the ileum of the lamb, before colostrum consumption, reported a lower level of expression in C lambs at 1 h[12]. The increase in *B2M* gene expression, from 1 to 24 h postpartum in control lambs, coincides with the ingestion of colostrum and is in agreement with previous work which found that *B2M* was up-regulated in the mammary gland during the period of active IgG transfer[32]. Despite the enhanced expression of *B2M* at 1 h[12] a similar pattern in expression was not observed in I-supplemented progeny over time. Thus, it would be beneficial to examine the catabolism of IgG along the digestive tract of newborn lambs in future experiments.

Colostrum intake promotes dramatic morphological and functional changes in the gastrointestinal tract of neonates including epithelial cell proliferation, migration and differentiation[54]. In addition, the influence of the thyroid hormones on cellular differentiation, growth and development in the fetus and neonate[55] has resulted in previous authors re-examining the impact of the thyroid hormones on the development and maturation of the gastrointestinal tract[12,55]. Following maternal supplementation with I, negative changes in the intestinal architecture of the lamb were observed (decreased VH and VH: CD ratio in the ileum), in the present study. Villi are fundamental components of the digestive tract and their geometry acts as an indicator of the absorptive capacity of the small intestine[56]. Hence, the VH:CD ratio has been identified as a useful parameter in the evaluation of intestinal health[57], with previous work reporting that the VH:CD ratio steadily increases in the lamb postnatally[58]. This is inconsistent with results obtained from the progeny of the I-supplemented ewes where a reduction in the VH:CD ratio was observed at 24 h postpartum and is in accordance with the decline in the absorption of serum IgG.

To date, the decline in serum IgG concentration following maternal supplementation with excess I has not been investigated alongside the neonatal lambs' response to infection postpartum. The increasing prevalence of GIN infections and the evolution of anthelminthic resistant nematodes has increased the use of chemoprophylaxis in sheep production systems[59,60]; hence, there is growing interest in developing alternative parasite control strategies. *N. battus* is one of the earliest nematodes to cause infection in the growing lamb, establishing itself in the host animal from 6 weeks of age[61] because of a temporary lapse in the immune-competence of the dam around the period of late pregnancy and early lactation[62].

Strategies which promote a robust immune response are therefore crucial to enhancing both the health and survival of the young lamb with regard to nematode infections[62]. Infection of growing lambs with GIN causes significant production losses through sub-optimal feed intake and nutrient utilisation[3,62]. Coinciding with a decline in $FEC_N$, animals born to I-supplemented ewes had higher growth rates from days 56 to 98 postpartum. Interestingly, results obtained from the present study show that lambs from I-supplemented ewes had an overall reduction in $FEC_N$, which was also evident on d 42 postpartum. This coincides with a rise in serum IgG concentrations, which were higher on day 70, among these lambs. Therefore, it may be hypothesised that the progeny of the I-supplemented ewes were primed for a heightened response to *N. battus* infection due to FPT in the neonatal period and the requirement to begin producing antibodies at an earlier age *v.* C lambs that had increased serum IgG concentrations in the postpartum period. Alternatively, lambs born to I-supplemented ewes may have been exposed to higher concentrations of I in the colostrum and milk during the postpartum period; thus proving toxic to *N. battus* in the gastrointestinal tract of the growing lamb.

In conclusion, the data presented in this study indicates that maternal supplementation with excess I during the late gestation period reduces serum IgG concentrations in the lamb; a decline that was evident up to day 28 postpartum. This FPT of IgG may be due in part to impaired intestinal integrity and down-regulation of *B2M*; which has the potential to reduce the total area available for absorption and increase the degradation of IgG in the intestine. Alternatively, the gene expression data from the duodenum indicate the possibility of recycling the IgG back into the intestinal lumen; however, further investigation into this is merited. Collectively, the results are mediated by the decline in $T_3$ concentrations and a down-regulation of *THRB* in the ileum. They provide new insights into the mechanisms governing FPT in the progeny of I-supplemented dams and highlight the effect this has on the lambs' own ability to produce IgG and subsequent positive responses to *N. battus* infection.

## Acknowledgements

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