



## Does iodine supplementation of the prepartum dairy cow diet affect serum immunoglobulin G concentration, iodine, and health status of the calf?

M. Conneely,\*† D. P. Berry,\* R. Sayers,\* J. P. Murphy,\* M. L. Doherty,† I. Lorenz,† and E. Kennedy\*<sup>1</sup>

\*Animal & Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland

†School of Veterinary Medicine, University College Dublin, Dublin 4, Ireland

### ABSTRACT

Absorption of adequate IgG from colostrum is critical to provide the newborn calf with adequate immunological protection and resistance to disease. Excessive iodine supplementation of the prepartum ewe reduces IgG absorption of her offspring; it is possible that excessive iodine supplementation of the prepartum dairy cow may similarly impair the ability of the calf to acquire immunological protection. The objectives of this study were to determine whether the iodine status, health status, and ability of calves to absorb IgG from colostrum were affected by prepartum iodine supplementation strategies of their dams. Dairy cows ( $n = 127$ ) received one of the following levels of iodine supplementation precalving: 15 mg of iodine/kg of dietary dry matter (DM) (HI); no additional iodine supplementation (MI); 5 mg/kg of dietary DM (SI); and 15 mg of iodine/kg of DM for the first 3.5 wk of the precalving period and no additional supplementation for the second 3.5 wk (HMI). Calves were assigned to 1 of 6 experimental treatments, based on the prepartum iodine supplementation treatment of their dam and the precalving treatment group of the cows from which the colostrum fed was obtained: (1) HI\_HI: born to HI dams, fed HI colostrum (i.e., colostrum produced by cows in the HI group); (2) MI\_MI: born to MI dams, fed MI colostrum; (3) SI\_SI: born to SI dams, fed SI colostrum; (4) HI\_MI: born to HI dams, fed MI colostrum; (5) MI\_HI: born to MI dams, fed HI colostrum; and (6) HMI\_HMI: born to HMI dams, fed HMI colostrum. Concentration of calf serum IgG and plasma inorganic iodine (PII) was measured at 0 and 24 h of age. Apparent efficiency of absorption for IgG was determined. Health scores were assigned to calves twice weekly and all episodes of disease were recorded. Cow experimental treatment group affected calf PII at 0 h of age; the PII of calves born to HI dams (987.2  $\mu\text{g/L}$ ) was greater than that of calves born to MI dams (510.1  $\mu\text{g/L}$ ), SI (585.2  $\mu\text{g/L}$ ),

and HMI dams (692.9  $\mu\text{g/L}$ ). Calf experimental treatment group affected calf PII at 24 h of age; the PII of HI\_HI (1,259.2  $\mu\text{g/L}$ ) and HI\_MI (1,177.8  $\mu\text{g/L}$ ) calves was greater than MI\_MI (240.7  $\mu\text{g/L}$ ), SI\_SI (302.2  $\mu\text{g/L}$ ), HMI\_HMI (320.7  $\mu\text{g/L}$ ), and MI\_HI (216.3  $\mu\text{g/L}$ ) calves. No effect of experimental treatment was observed on the concentration of IgG measured in calf serum at 24 h of age, or on apparent efficiency of absorption. Experimental treatment had no effect on the likelihood of a calf being assigned a worse nasal, eye and ear, cough, or fecal score within the study period, nor did it affect the probability of a calf receiving treatment for a disease a greater number of times. Prepartum iodine supplementation of cows at 15 mg/kg of DM increased the iodine levels in their calves at birth and 24 h of age, but did not affect their ability to absorb IgG from colostrum. Supplementation with iodine above the minimum requirements established by the National Research Council was unnecessary to ensure appropriate iodine levels in calves at birth.

**Key words:** calf, colostrum, immunoglobulin G, iodine

### INTRODUCTION

Absorption of sufficient IgG provides the neonatal calf with vital immunological resistance to combat disease and has been shown to have numerous short- and long-term benefits. These benefits include reduced risk of pre- and postweaning morbidity and mortality, improved rate of weight gain and feed efficiency, as well as greater milk production and longevity within the herd (Robison et al., 1988; DeNise et al., 1989). The transfer of immunoglobulins from the dam to the neonatal calf is termed passive transfer. Adequate passive transfer is said to have occurred if the IgG level in the serum of the calf is  $\geq 10$  g/L when sampled between 24 and 48 h of age (Besser et al., 1991).

Recent studies and surveys indicate inadequate transfer of passive immunity occurs in a high proportion of neonatal calves internationally (USDA, 2007; Trotz-Williams et al., 2008; DAFM, 2011; Panousis et al., 2013). This suggests that inadequate absorption of IgG from colostrum may be an important contributory

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<sup>1</sup>Corresponding author: Emer.Kennedy@teagasc.ie

factor to the high mortality rate of calves worldwide, including Ireland (5.1% calves dead in the first 12 mo of life, excluding stillbirths; DAFM, 2012). Failure of passive transfer in the neonatal calf may occur for several reasons, including (1) ingestion of an insufficient volume of colostrum, (2) ingestion of colostrum during the period when the neonatal gut is no longer capable of absorbing IgG, and (3) ingestion of colostrum containing an inadequate concentration of IgG (Godden, 2008). Other factors may also reduce the capacity of the neonatal intestine to absorb IgG; previous studies have demonstrated that high levels of iodine fed to ewes in late pregnancy can impair the ability of their lambs to absorb IgG from colostrum, due to a preprogrammed decrease in absorption ability (Boland et al., 2004; Crosby et al., 2004; Boland et al., 2008).

Supplementation of the prepartum dairy cow diet with iodine is standard practice across most dairy farms. However, in many cases it is possible that cows are being supplemented with a level higher than the maintenance iodine requirement established by the NRC (requirement of the pregnant nonlactating cow is 0.33 mg of iodine/kg of dietary DM; NRC 2001), although definitive data are lacking at present to confirm this. Further supplementation is unnecessary and has the potential to cause a state of toxicity and consequent harmful effects, including an increase in reproductive disorders (Paulíková et al., 2002), excessive nasal and ocular discharge, salivation, decreased milk production, coughing, dry and scaly coats (Olson et al., 1984), and suppression of the immune system (Hillman and Curtis, 1980). Furthermore, excessive iodine supplementation of the prepartum cow may negatively affect the transfer of immunity from dam to calf if the same phenomenon that has been demonstrated to occur in sheep (Boland et al., 2004; Crosby et al., 2004; Boland et al., 2008) occurs in cattle.

Whether inadequate absorption of IgG results from over-supplementation of iodine in the pregnant cow is presently unclear. Gilles et al. (2009) and Rose et al. (2012) reported no reduced absorption of IgG in calves of dams supplemented with iodine; however, both these studies used a bolus as a means of iodine supplementation and limited studies have examined the effects of providing mineral supplementation via a powder, which delivers a specific quantity of iodine on a daily basis. In addition, varying levels of iodine supplementation used in the studies by Gilles et al. (2009) and Rose et al. (2012) means it remains uncertain whether the same phenomenon that has been demonstrated in sheep also occurs in cattle.

Therefore, the objectives of the present study were to determine (1) the effects of offering varying levels of supplementary iodine to cows during the last 7 wk

of pregnancy on the serum IgG concentration, plasma inorganic iodine (PII) concentration, and health parameters of their calves; (2) the effects of timing of iodine supplementation of cows and heifers during the precalving period on the serum IgG concentration, PII concentration, and health parameters of their calves; and (3) whether any reduction in the serum IgG concentration of the calves was mediated through a pre-programming of the calf with an impaired absorptive ability or through an effect on the colostrum itself.

## MATERIALS AND METHODS

The current study was conducted from December 13, 2011, to June 19, 2012, at the Teagasc Moorepark Research farm located in County Cork, in southern Ireland (52°9'N, 8°16'W). One hundred twenty-seven dairy cows were used to provide calves for the study. In total, 48 first parity animals were used, and 37, 18, 11, and 13 cows in their second, third, fourth, and fifth or greater parity, respectively.

### *Iodine Supplementation of Cows and Heifers*

Seven weeks before their respective calving due date (i.e., at dry-off for cows), animals were stratified based on breed, parity, and calving date and randomly assigned, within stratum, to 1 of 4 precalving treatment groups. The 4 groups were based on the level of iodine supplementation of the precalving diet as (1) high iodine supplementation (**HI**; 15 mg of iodine/kg of dietary DM, 3 times the current European Union maximum permitted limit); (2) maintenance iodine (**MI**; no additional iodine supplementation, although the diet was formulated to ensure that maintenance iodine requirement was satisfied; the maintenance iodine requirement of the pregnant nonlactating cow is 0.33 mg of iodine/kg of dietary DM; NRC 2001); (3) standard iodine (**SI**; supplementation at 5 mg of iodine/kg of dietary DM, the current European Union maximum permitted level of iodine); and (4) high to maintenance iodine (**HMI**; supplemented at the HI level for the first 3.5 wk of the dry period and the MI level for the last 3.5 wk; Table 1).

### *Cow Management and Diet*

Following dry-off, cows were housed in a cubicle shed in their separate treatment groups and fed a TMR diet ad libitum, which consisted of 3 kg of dry cow concentrate, 2 kg of straw, 33 to 40 kg of silage (depending on the DM%), and 0.25 g of molasses per cow per day (on a fresh weight basis). Fresh TMR was offered daily and the refused feed was removed every second

**Table 1.** Level of iodine provided by the basal diet and supplementation, total iodine intake, and relationship to requirements and toxicity for cows of the high iodine (HI), maintenance iodine (MI), standard iodine (SI), and high to maintenance (HMI) experimental treatments

Cow experimental treatment	Level of iodine supplementation (mg/kg of DM)	Iodine from basal diet (mg/kg of DM)	Total daily iodine intake <sup>1</sup> (mg/d)	Relationship to requirement <sup>2</sup> (times requirement)	Relationship to toxicity <sup>3</sup> (times toxicity)
HI	15	0.33	165.7	46	3.3
MI	—	0.33	3.6	1	1/14
SI	5	0.33	57.6	16	1.15
HMI					
First 3.5 wk of the dry period	15	0.33	165.7	46	3.3
Second 3.5 wk of the dry period	—	0.33	3.6	1	1/14

<sup>1</sup>Total iodine intake was calculated assuming an average cow BW of 600 kg and a DMI of 1.8% of BW per cow per day.

<sup>2</sup>Toxicity level of iodine is 50 mg/d (NRC, 2001).

<sup>3</sup>The iodine requirement of the pregnant, nonlactating cow is 0.33 mg/kg of DM.

day. Late gestation cows were moved into individual straw-bedded calving pens before calving. Mineral powders were specially formulated (Table 2) to contain the specified quantity of iodine for each experimental group to ensure that the desired quantities of iodine and other minerals could be delivered when offered at a rate of 100 g/cow per day (Multitrace Pre-calver, Inform Nutrition Ireland, Whites Cross, Cork, Ireland). The mineral powder was topically applied to the TMR twice daily. Fresh water was available at all times.

### Experimental Treatments of Calves

The resulting calf study population consisted of 128 dairy calves (59 female and 69 male) consisting of 68 Holstein-Friesian, 50 Jersey × Holstein-Friesian, and 10 Holstein-Friesian × Norwegian Red. The median date of birth of the calves was February 6; mean calf BW at birth was 35.9 kg (SD 6.1). Calves were assigned to 1 of 6 experimental treatment groups (Figure 1) based on the level of prepartum iodine supplementation of their dam and the origin of the colostrum they received (i.e., the precalving treatment group of the cow or cows from which the colostrum fed was obtained). The experimental treatments were: (1) high iodine (**HI\_HI**; born to HI dams, fed HI colostrum; i.e., colostrum produced by cows in the HI group); (2) maintenance iodine (**MI\_**

**MI**; born to MI dams, fed MI colostrum); (3) standard iodine (**SI\_SI**; born to SI dams, fed SI colostrum); (4) high iodine crossover (**HI\_MI**; born to HI dams, fed MI colostrum); (5) maintenance I crossover (**MI\_HI**; born to MI dams, fed HI colostrum); and (6) high to maintenance I (**HMI\_HMI**; born to HMI dams, fed HMI colostrum).

### Colostrum Feeding and Management of Calves at Calving

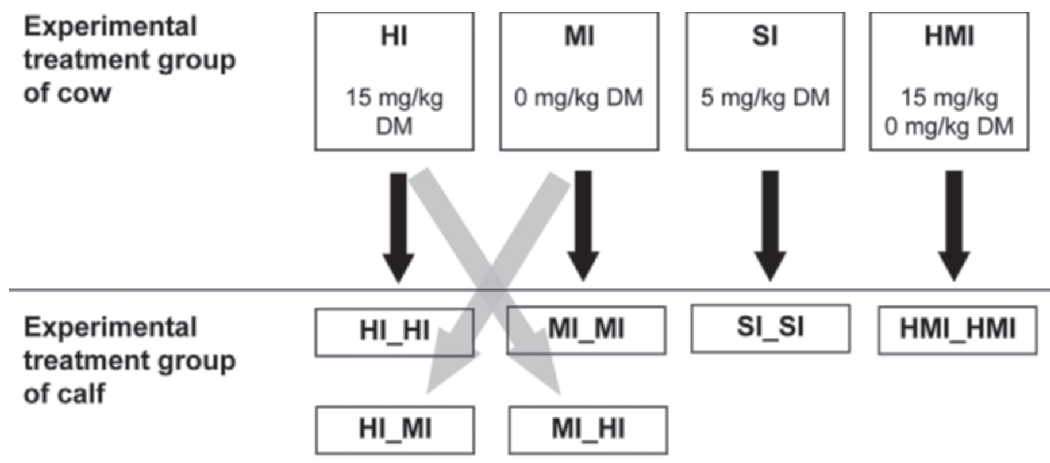
All calving events were attended and observed by competent and trained personnel. To ensure the calf did not suckle its dam before feeding of colostrum or blood sampling (detailed below), the calf was separated from the dam before it became ambulatory. Each calf was weighed (TruTest XR 3000, Tru-test Limited, Auckland, New Zealand) and a blood sample was obtained (detailed below). The calf was then fed colostrum (8.5% of BW) according to its experimental treatment group.

If possible, the colostrum fed to each calf at the initial feed following birth was colostrum obtained from the first milking postcalving of its own dam (except for calves assigned to the HI\_MI or MI\_HI treatments). If it was not possible to feed the calf colostrum from its own dam, the calf was fed colostrum obtained from one of the other recently calved cows from the same iodine supplementation group. All initial feeds of colostrum were administered via an oroesophageal tube.

Each calf was then placed in an individual pen, measuring 0.8 × 1.2 m, where it remained for 3 d and received 4 feeds of transition milk, which were pooled from the second milking postcalving of recently calved cows from their respective treatment group. All subsequent feeds were given by nipple bottle, and the quantity fed at each feeding was 2 L. Pooled transition milk was retained for a maximum of 2 d, after which it was discarded and a new batch was created. The subsequent transition milk feeds were given at the next standard feeding times (0800 and 1500 h) unless a calf was born

**Table 2.** Composition of mineral supplement used in dry-cow diet

Item	Quantity (per 100 g of mineral supplement)
Magnesium (g)	17
Iodine	Varied according to treatment
Selenium (mg)	6
Copper (mg)	400
Manganese (mg)	125
Cobalt (mg)	9.9
Zinc (mg)	500
Vitamin A (IU)	27,500
Vitamin D (IU)	750
Vitamin E (IU)	50



**Figure 1.** Division of cows and calves into experimental treatment groups. Arrows indicate the origin of the colostrum fed to each calf experimental treatment group. HI = high iodine; MI = maintenance iodine; SI = standard iodine; HMI = high to maintenance iodine; HI\_HI = born to HI dams, fed HI colostrum; MI\_MI = born to MI dams, fed MI colostrum; SI\_SI = born to SI dams, fed SI colostrum; HI\_MI = born to HI dams, fed MI colostrum; MI\_HI = born to MI dams, fed HI colostrum; HMI\_HMI = born to HMI dams, fed HMI colostrum.

within the 3 h before the next standard feeding time. In this case, the subsequent transition milk feed was given at the following standard feeding time. A sample from both the first milking colostrum and the transition milk fed to each calf was taken for subsequent IgG analysis. These samples were stored at  $-20^{\circ}\text{C}$  until analysis.

#### Management of Calves Postcolostrum and Transition Milk Feeding

Following feeding of transition milk, calves were moved to a group pen of 16 animals; the largest difference in birth date within group was 2 wk. Bulls and heifers were housed in separate pens. Heifer calves remained in the group pen until they were turned out to pasture at 4 wk of age, where they grazed in groups of 16 calves per 0.8-ha paddock. Bull calves were sold between 3 and 4 wk of age.

Calves were fed whole milk twice a day at a daily rate of 15% of birth BW/calf until 3 wk of age, when they received the same quantity in a once-daily feed. Heifer calves were weaned once they had attained specific target weights for their breed (90 kg for Holstein-Friesian, 80 kg for Holstein-Friesian  $\times$  Norwegian Red, 75 kg for Jersey  $\times$  Holstein-Friesian). The calves were then removed from the group of calves with which they were grazing and gradually weaned during the following week. All calves were managed similarly postweaning and had full-time access to pasture plus 1 kg (fresh weight) of supplementary concentrate feed offered per day.

Fresh water was available at all times and ad libitum concentrate and hay were offered from 4 d of age. The

general health of the calves was monitored twice daily by an on-site veterinarian; any animal that became ill received the appropriate care and veterinary treatment as required.

#### Data Collection and Analysis

**Collection of Calf Serum and Plasma.** Two 6-mL blood samples were taken from the jugular vein of each calf within 1 h of birth, before feeding colostrum (0 h): 1 sample into a plain serum tube and 1 sample into a lithium heparin tube (BD Vacutainer, Lagenbach, Germany). Two further blood samples were taken again from each calf at 24 h of age. Blood collected in lithium heparin tubes was separated by centrifugation at  $3,500 \times g$  for 30 min at  $4^{\circ}\text{C}$  within 2 h of collection to obtain plasma; samples collected in plain serum tubes were refrigerated for 24 h before serum was separated by centrifugation at  $3,500 \times g$  for 30 min at  $4^{\circ}\text{C}$ . Both serum and plasma were frozen at  $-20^{\circ}\text{C}$  before determination of serum IgG and plasma inorganic iodine concentration determination.

**Laboratory Analysis of Serum, Colostrum, and Plasma.** The IgG concentration in serum and colostrum was determined by the ELISA method (Bovine IgG ELISA Kit Cat. No. 8010, Alpha Diagnostic International, San Antonio, TX). Samples were assayed in duplicate with an interassay CV of 0.15. The concentration of IgG in 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. The PII concentration was determined by using ion-exchange chromatography (Aumont and Tressol, 1987).

**Collection of Calf Health Data.** Individual animal health scores were assigned to calves on a twice-weekly basis by a single veterinarian. Heifer calves were scored from birth until weaning at approximately 12 wk of age; scores were assigned to male calves from birth until 3 to 4 wk of age. Health scores were assigned using a calf health-scoring system developed by the School of Veterinary Medicine, University of Wisconsin-Madison ([http://www.vetmed.wisc.edu/dms/fapm/fapmtools/8calf/calf\\_health\\_scoring\\_chart.pdf](http://www.vetmed.wisc.edu/dms/fapm/fapmtools/8calf/calf_health_scoring_chart.pdf)). Calves were scored on 4 different aspects of health: nasal, eye and ear, fecal, and cough. Each individual aspect received a score from zero to 3; zero representing normal and 3 representing the most severely affected.

**Collection of Calf Disease Data.** All calves were examined twice daily by an on-farm veterinarian. Assessment was made of general demeanor, fecal consistency, and respiratory rate. Calves were examined to determine the presence of a cough and nasal and ocular discharge. Any calf that was exhibiting any abnormal clinical sign was subjected to a complete clinical examination that included determination of temperature and auscultation of the lungs. Any animal that became ill received the appropriate care and veterinary treatment, if required, and all episodes of disease and treatments were recorded. The decision as to whether a disease episode was severe enough to necessitate treatment and the type and duration of treatment required was made by the farm veterinarian in consultation with the farm manager. Calves that died during the study period ( $n = 6$ ) were subject to routine necropsy examination.

**Determination of Apparent Efficiency of Absorption.** Apparent efficiency of absorption (AEA) estimates the efficiency of immunoglobulin absorption before cessation of intestinal absorption of immunoglobulins (Quigley et al., 1998). Apparent efficiency of absorption of IgG was determined as previously described (Quigley et al., 1998, 2002) using the following formula:

$$\text{AEA} = \left[ \frac{\text{serum IgG (g/L)} \times \text{plasma volume (L)}}{\text{IgG intake (g)}} \right] \times 100.$$

The plasma volume was calculated as plasma volume =  $0.089 \times (\text{BW at birth, kg})$  (Quigley et al., 1998).

### Data Editing and Statistical Analysis

**IgG of Colostrum Fed to Calves.** A fixed effects model in PROC GLM (SAS version 9.3, SAS Institute

Inc., Cary, NC) was used to establish whether the IgG concentration of the colostrum fed to calves at the first feeding after birth or the total mass of IgG fed to each calf in the first 24 h of life differed between experimental treatment groups. The independent variable in both cases was experimental treatment group of calf. Concentration of IgG in colostrum fed to calves was normally distributed, as was the total mass of IgG fed to each calf in the first 24 h of life.

**Calf Serum IgG and AEA.** Concentration of IgG in the calf serum was normally distributed. The AEA was calculated for 117 of the 128 calves; data required to calculate AEA were missing for 11 of the 128 calves, so these were excluded from the analysis. Mixed models in PROC MIXED (SAS version 9.3) were used to determine the effect of experimental treatment on serum IgG concentrations at 0 and 24 h of age. Independent variables considered for inclusion in the model included experimental treatment group of the calf and age of the calf when serum was sampled (0 and 24 h). Interactions between the experimental treatment group of calf and age of calf were also considered in the model. Calf was included as a repeated effect and heterogeneous variances were assumed among records. A fixed effects model in PROC GLM (SAS version 9.3) was used to establish whether the experimental treatment group of calf affected the AEA of IgG at 24 h of age. The independent variable in the model was experimental treatment group of calf.

Because feeding of the subsequent transition milk feeds took place at fixed times during the day (0800 and 1500 h), and because of the spread in the actual times of birth of each calf, variation existed in the number of subsequent transition milk feeds each calf received before cessation of IgG absorption at 24 h of age depending on their time of birth. Thus, the time of birth of the calf was considered as a covariate also. Time of birth of the calf was categorized as (1) calf born between 0500 and 0800 h, next transition milk feed at 1500 h; (2) calf born between 0800 and 1200 h, next transition milk feed at 1500 h; (3) calf born between 1200 and 1500 h, next transition milk feed at 0800 h; and (4) calf born between 1500 and 0500 h, next transition milk feed at 0800. Time of birth of the calf was not associated with serum IgG concentration or AEA, and thus was not retained in the final models.

**Calf PII.** Concentration of iodine in calf plasma was normally distributed. Mixed models in PROC MIXED (SAS version 9.3) were used to determine the effect of experimental treatment on PII concentrations at 0 and 24 h of age. Independent variables considered for inclusion in the model included experimental treatment group of the calf and age of the calf when plasma was sampled (0 and 24 h). Interactions between experimen-

tal treatment group of calf and age of calf were also considered in the model. Calf was included as a repeated effect and heterogeneous variances were assumed among records. Orthogonal contrasts were used to compare (1) the HI\_HI and HI\_MI treatments combined, against the SI\_SI treatment; (2) the HI\_HI and HI\_MI treatments combined, against the HMI\_HMI treatment; (3) the HI\_HI and HI\_MI treatments combined, against the MI\_MI and MI\_HI treatments combined; (4) the MI\_MI and MI\_HI treatments combined, against the SI\_SI treatment; and (5) the MI\_MI and MI\_HI treatments combined, against the HMI\_HMI treatment.

**Calf Health Scores.** Five calves left the farm before commencement of health score recording, thus health score data were unavailable for these calves. A total of 1,543 individual calf health scores from 123 calves were available for analysis. For each health aspect separately, the probability of a calf having a higher welfare score (i.e., worse score) within the study period was modeled by ordinal regression in PROC GENMOD (SAS version 9.3) utilizing a cumulative logit link function and a multinomial distribution. Calf was included as a repeated effect. The model included experimental treatment group of calf. The number of days a calf was present in the study before it was weaned or sold was also included as a covariate in the model. Significance was declared at  $P < 0.05$ .

**Disease.** Calf diseases recorded were pneumonia, diarrhea, and omphalophlebitis. A case of pneumonia was defined as a calf displaying increased respiratory rate and effort, pyrexia, copious bilateral nasal discharge, or repeated coughing (alone or in combination). A case of diarrhea was defined as a calf repeatedly passing loose or watery feces, with or without blood content. A case of omphalophlebitis was defined as a calf exhibiting a swollen and painful umbilicus, with or without exudation of pus. The number of times a calf was treated for a disease was recorded as never, once, twice, or 3 times. The probability of a calf receiving treatment for any disease a greater number of times within the study period was modeled with ordinal regression in PROC GENMOD (SAS version 9.3) utilizing a cumulative

logit link function and assuming a multinomial distribution of the data. Experimental treatment of calf was included in the model. The number of days a calf was present in the study before it was weaned or sold was also included as a covariate in the model.

## RESULTS

### *IgG Concentration of Colostrum of Cows and Colostrum Fed to Calves*

The IgG concentration of the colostrum fed to calves did not differ ( $P > 0.05$ ) between experimental groups. The mean concentration of IgG in the colostrum fed to all calves at the first feeding was 117.0 g/L (SD = 41.1 g/L). The mean total mass of IgG fed in the first 24 h of life to the calves in the 6 experimental treatment groups was 15.7 g/kg of BW (SD = 5.3) and did not differ ( $P = 0.257$ ) between experimental groups.

### *Serum IgG and AEA*

Age of calf when serum was sampled affected the concentration of IgG ( $P < 0.001$ ). Mean concentration of IgG in serum at 0 h was 0.34 g/L (SE = 0.66) and at 24 h of age was 34.1 g/L (SE = 0.66). No effect ( $P = 0.227$ ) of experimental treatment was observed on the concentration of IgG measured in serum (Table 3), and no effect ( $P = 0.319$ ) of age of calf  $\times$  treatment interaction was noted. The AEA did not differ between treatment groups ( $P > 0.05$ ).

### *PII*

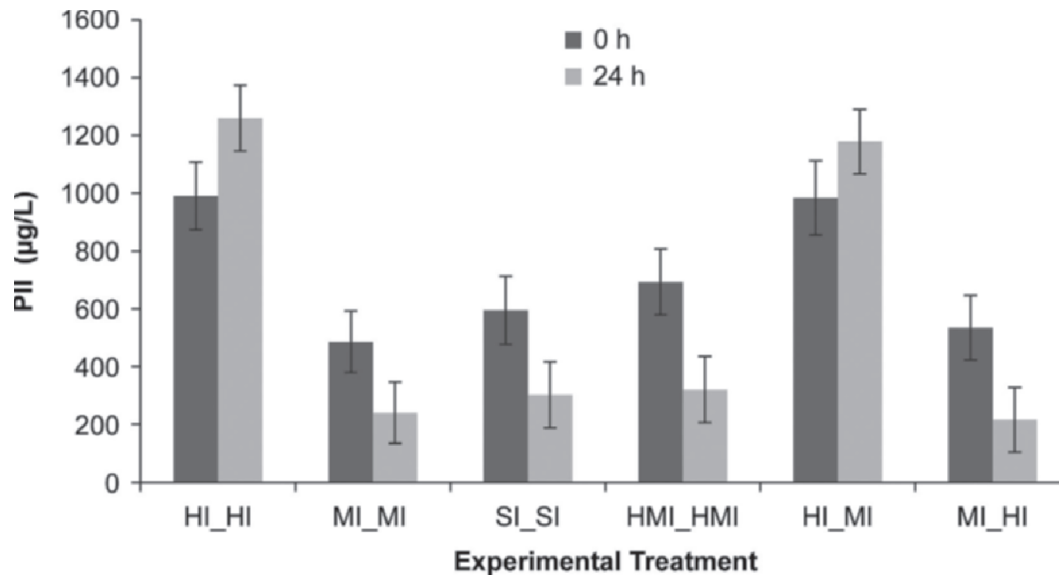
The orthogonal contrasts show that calf PII at 0 h of age was affected by cow experimental treatment group ( $P < 0.05$ ). The PII of calves born to dams in the HI treatment group (i.e., the PII of the HI\_HI and HI\_MI calf treatment groups combined; 987.2  $\mu$ g/L) was greater than the PII of calves born to dams in the MI treatment group (i.e., the PII of the MI\_MI and MI\_HI calf treatment groups combined; 510.1  $\mu$ g/L;  $P < 0.01$ ),

**Table 3.** Mean serum IgG (g/L) concentrations at 0 and 24 h of age for calves of the different experimental treatment groups

Item	Time	Experimental treatment <sup>1</sup>						<i>P</i> -value <sup>2</sup>		
		HI_HI	MI_MI	SI_SI	HMI_HMI	HI_MI	MI_HI	T	A	T $\times$ A
IgG (g/L)	0 h	0.5 (0.1)	0.3 (0.1)	0.3 (0.1)	0.2 (0.1)	0.2 (0.1)	0.5 (0.1)	0.227	<0.001	0.319
	24 h	38.9 (2.3)	34.6 (2.2)	32.7 (2.3)	33.3 (2.4)	31.4 (2.3)	33.6 (2.3)			

<sup>1</sup>SE are in parentheses. HI\_HI = high iodine; born to HI dams, fed HI colostrum; MI\_MI = maintenance iodine; born to MI dams, fed MI colostrum; SI\_SI = standard iodine; born to SI dams, fed SI colostrum; HMI\_HMI = high to maintenance iodine; born to HMI calves, fed HMI colostrum; HI\_MI = high iodine crossover; born to HI dams, fed MI colostrum; MI\_HI = maintenance iodine crossover; born to MI dams, fed HI colostrum.

<sup>2</sup>T = effect of experimental treatment group; A = calf age; T  $\times$  A = interaction of calf experimental treatment group and age.



**Figure 2.** Mean plasma inorganic iodine (PII;  $\mu\text{g/L}$ ) concentrations at 0 and 24 h of age for calves of the HI\_HI, MI\_MI, SI\_SI, HMI\_HMI, HI\_MI and MI\_HI experimental treatment groups. HI = high iodine; MI = maintenance iodine; SI = standard iodine; HMI = high to maintenance iodine; HI\_HI = born to HI dams, fed HI colostrum; MI\_MI = born to MI dams, fed MI colostrum; SI\_SI = born to SI dams, fed SI colostrum; HI\_MI = born to HI dams, fed MI colostrum; MI\_HI = born to MI dams, fed HI colostrum; HMI\_HMI = born to HMI dams, fed HMI colostrum.

as well as that of calves born to dams in the SI (585.2  $\mu\text{g/L}$ ;  $P < 0.001$ ) and HMI treatment groups (692.9  $\mu\text{g/L}$ ;  $P < 0.05$ ). Calf PII at 24 h of age was affected by cow experimental treatment group ( $P < 0.05$ ). The PII of calves born to dams in the HI treatment group (i.e., the PII of the HI\_HI and HI\_MI calf treatment groups combined; 1218.5  $\mu\text{g/L}$ ) was greater than the PII of calves born to dams in the MI treatment group (i.e., the PII of the MI\_MI and MI\_HI calf treatment groups combined; 228.5  $\mu\text{g/L}$ ;  $P < 0.001$ ), as well as that of calves born to dams in the SI (302.2  $\mu\text{g/L}$ ;  $P < 0.001$ ) and HMI treatment groups (320.7  $\mu\text{g/L}$ ;  $P < 0.001$ ).

The effect of calf experimental treatment group on PII differed ( $P < 0.05$ ) with the age of the calf (Figure 2). At 0 h of age, calves in the HI\_HI treatment group had a greater concentration of PII (990.7  $\mu\text{g/L}$ ) than calves in the MI\_MI (485.4  $\mu\text{g/L}$ ;  $P < 0.001$ ), SI\_SI (585.2  $\mu\text{g/L}$ ;  $P < 0.05$ ) and MI\_HI treatment groups (534.6  $\mu\text{g/L}$ ;  $P < 0.01$ ), and tended to have a greater concentration of PII than calves in the HMI\_HMI treatment group (692.9  $\mu\text{g/L}$ ;  $P = 0.07$ ). Similarly, calves in the HI\_MI treatment group had a greater concentration of PII (983.6  $\mu\text{g/L}$ ) than calves in the MI\_MI, SI\_SI and MI\_HI treatment groups ( $P < 0.001$ ,  $< 0.05$ , and  $< 0.001$ , respectively) and tended ( $P = 0.09$ ) to have a greater concentration of PII than calves in the HMI\_HMI treatment group. No difference was observed between the PII of calves in the HI\_HI treatment group and calves in the HI\_MI treatment group ( $P = 0.967$ ).

At 24 h of age, the PII of calves in MI\_MI, SI\_SI, HMI\_HMI, and MI\_HI treatment groups decreased, but the PII of calves in the HI\_HI and HI\_MI treatment groups did not. At 24 h of age, calves in the HI\_HI treatment group had a greater concentration of PII (1218.5  $\mu\text{g/L}$ ) than calves in the MI\_MI (240.7  $\mu\text{g/L}$ ;  $P < 0.001$ ), SI\_SI (302.2  $\mu\text{g/L}$ ;  $P < 0.001$ ), HMI\_HMI (320.7  $\mu\text{g/L}$ ;  $P < 0.001$ ), and MI\_HI treatment groups (216.3  $\mu\text{g/L}$ ;  $P < 0.001$ ). Similarly, calves in the HI\_MI treatment group had a greater concentration of PII (1,177.8  $\mu\text{g/L}$ ) than calves in the MI\_MI, SI\_SI, MI\_HI, and HMI\_HMI treatment groups ( $P < 0.001$  for all). No difference was noted between the PII of calves in the HI\_HI treatment group and calves in the HI\_MI treatment group ( $P = 0.610$ ).

### Disease

A total of 50 disease episodes (i.e., calf received treatment for a disease) were recorded between January 24 and June 19, 2012. We observed 25 cases of pneumonia, 21 cases of diarrhea, 3 of omphalophlebitis (navel-ill), and 1 calf developed septicaemia. Twenty-seven calves had at least 1 disease episode. Experimental treatment of calf did not affect the probability of a calf receiving treatment for a disease a greater number of times ( $P = 0.664$ ). Six calves died during the study period: 3 calves from the MI\_HI treatment, 2 calves from the HI\_HI treatment, and 1 calf from the SI\_SI treatment. One calf from the HI\_HI treatment died of pneumonia and 1

died from complication of omphalophlebitis. Two calves from the MI\_HI treatment died of pneumonia and 1 calf died of diarrhea. The calf from the SL\_SI treatment was euthanized because it had atresia coli.

### Health Scores

The overall prevalence of scores indicating normal health was high; 81.5, 87.2, 92.4, and 92.7% of the scores were zero (i.e., normal health) for nasal, eye and ear, cough, and fecal consistency aspects of health. Scores of 2 and 3 were assigned for 14 and 4% of nasal scores, 10 and 2% of eye and ear scores, 4 and 3% of cough scores, and 5 and 2% of fecal consistency scores, respectively. Less than 1% of scores assigned were indicative of severely abnormal health (i.e., score of 3). Experimental treatment had no effect on the likelihood of a calf being assigned a worse nasal ( $P = 0.366$ ), eye and ear ( $P = 0.704$ ), cough ( $P = 0.875$ ), or fecal ( $P = 0.958$ ) score within the study period.

## DISCUSSION

### Effect of Iodine Supplementation of Cows on IgG Absorption in Calves

In the present study, feeding cows varying levels of supplementary iodine during the 7-wk precalving period had no effect on the IgG absorption efficiency or level of IgG measured in the serum of their calves at 24 h of age. This is a similar result to those reported in previous studies undertaken in cattle; Rose et al. (2012) reported that the administration of a bolus containing 6,800 mg of iodine to 25 nonlactating Holstein-Friesian dairy cows approximately 8 wk before parturition had no effect on the plasma concentration of IgG or on the efficiency of absorption of IgG by calves during the first day of life. Serum IgG concentration at 24 h of age of calves born to supplemented dams (15.5 g/L) was not different to that of calves born to unsupplemented dams (13.4 g/L). Similarly, Gilles et al. (2009) reported no difference in IgG concentration measured in calf serum at 24 to 36 h of age between calves born to dams supplemented with a bolus containing 1,200 mg of iodine 20 to 35 d before the parturition due date (25.1 g/L) and calves born to unsupplemented cows (19.8 g/L). The present study is the first, to our knowledge, that has examined the effects of offering bovine animals varying levels of iodine supplementation using a mineral powder. The results of the present study and the studies mentioned herein are in contrast to previous research carried out in sheep, which reported that feeding a high level of iodine in the prepartum diet to ewes can indeed reduce serum IgG concentration and IgG

absorption efficiency in the lamb (Boland et al., 2004, 2005a,b; Crosby et al., 2004).

It is unclear at present why this discrepancy in results between cows and sheep exists. Prior to the present study, it was plausible that the level of iodine fed in the studies by Gilles et al. (2009) and Rose et al. (2012) were simply not high enough to cause the effect on serum IgG concentration reported in sheep. The levels of iodine delivered to the cows in the studies by Rose et al. (2012) and Gilles et al. (2009) on a daily basis by the bolus [56.7 and 43.6 mg/d (equivalent to 0.44 and 0.36 mg/kg<sup>0.75</sup> per day, respectively)] were lower than the level previously demonstrated to cause a reduction in colostral IgG absorption in sheep (14.8 mg/d, equivalent to 0.62 mg/kg<sup>0.75</sup> per day; Rose et al., 2007). In fact, the level of iodine delivered to the cows in both studies was likely to have been much lower toward the end of the supplementation period, as the release of minerals from boluses appears not to be constant (Watson et al., 2012); rather, the release is much greater following initial insertion and much less as the boluses age. Rose et al. (2012) postulated that perhaps a similar reduction in the IgG absorption by the calves would have occurred had the cows in their study been supplemented with iodine at a greater rate. However, the cows in the HI precalving experimental treatment group in the present study were supplemented with 162 mg/d (equivalent to 1.34 mg/kg<sup>0.75</sup> per day), delivered at a consistent daily rate via a powder. This is over 2 times greater than the level shown to cause an effect in sheep per unit of metabolic live weight, and, yet, still did not have any effect on the IgG status of the calves.

It is possible that iodine exerts a different effect on the biological pathways that mediate the intestinal absorption of IgG in the neonate, specifically the thyroid hormones. Iodine is necessary for the synthesis of the thyroid hormones, thyroxine and triiodothyronine (**T3**), which regulate energy metabolism (NRC, 2001) and have numerous functions as regulators of cell activity and growth. Earlier work has indicated a relationship between the concentration of thyroid hormones in the immediate prepartum period and the transmission of IgG in the neonate; greater levels of T3 increase IgG absorption in both lambs (Boland et al., 2008) and calves (Slebodziński et al., 1995; Rose et al., 2012). However, whereas a high level of supplementary iodine offered to prepartum ewes reduced T3 in lambs (Boland et al., 2008), the same did not occur in calves (Rose et al., 2012). Thus, it appears that thyroid hormones are related to IgG absorption in both cows and sheep, but that iodine affects the concentration of thyroid hormones in sheep in a way that it perhaps does not in cattle. This is an area that warrants further detailed research.

Adequate passive transfer is critical to ensure the calf has adequate immune defenses to protect it from disease until development of its own immunity occurs at 3 to 4 wk of age (Godden, 2008). Given the high IgG concentration of the colostrum fed to calves in the present study (117 g/L), their high serum IgG level is to be expected, as concentration of IgG in colostrum is a critical factor in ensuring adequate absorption by the calf (Pritchett et al., 1991). Furthermore, all calves were fed a large volume of colostrum very early following birth; this also undoubtedly contributed to their high absorption of IgG, as both volume (Godden, 2008) and time of ingestion (Stott et al., 1979) of colostrum are important factors in determining the concentration of IgG achieved in calf serum. In the study by Rose et al. (2012), calves received colostrum of a lower IgG concentration (mean 74.5 g/L) and only received 4.5% of their BW at the first feed compared with the calves in the present study, who received 8.5%. The calves in the study of Rose et al. (2012) received an average total of 367 g of IgG within the first 24 h of life; calves in the present study received 53% more. The comparatively greater serum IgG concentration at 24 h in the calves of the present study is a reflection of the greater quantity of IgG available to the calves. Gilles et al. (2009) did not measure the IgG concentration of the colostrum that was fed to the calves in their study, or control the quantity of colostrum that was ingested.

It may be that a high level of iodine supplementation in the diet of parturient cows would have resulted in a reduction in absorption efficiency of IgG of their calves only when the amount of IgG available for absorption from colostrum was lower than that of the current study. In the present study, colostrum IgG concentration was not determined before feeding it to calves, and subsequent analysis revealed that the mean IgG delivered to the calves was high (an average of 563.1g of IgG was delivered to each calf). Approximately only 150 to 200 g of total IgG is required for adequate passive transfer in calves (Chigerwe et al., 2008). It is possible that AEA may have been affected at lower IgG concentrations, but the design of the current study did not allow for detection of this. This is an area that warrants further research.

In the present study, considering that no difference in serum IgG at 24 h of age was noted between experimental groups and, as such, all calves had the same degree of protection from disease, it is unsurprising that experimental treatment group of calf had no effect on any of the health scores measured, or on the likelihood of a calf receiving treatment for a disease during the study period. Boland et al. (2008) reported a greater level of upper tail or anal region fecal soiling in lambs born to dams supplemented with a high level of iodine

compared with lambs born to unsupplemented ewes. Lambs born to dams supplemented with a high level of iodine had a reduced serum IgG absorption efficiency and serum IgG concentration at 24 h, and it was suggested that the greater fecal soil scores were perhaps due to a breakdown in the digestive process in the gut of these lambs, resulting in an increased rate of passage of colostrum through the intestine of these lambs. No such response was observed in the present study.

Earlier work in sheep has demonstrated that the timing of the iodine supplementation is important in relation to reducing IgG absorption of the progeny (Boland et al. 2008), with a high level of supplementation during the final week alone sufficient to cause a reduction in absorption. In the present study, the timing of supplementation had no effect on the level of IgG absorption in the calf. If, as the results of the present study would suggest, iodine supplementation does not cause a reduction in the IgG absorption efficiency in cattle in the same way it has been documented in sheep, then the fact that the timing of supplementation had no effect is to be expected.

#### ***Effect of Iodine Supplementation of Cows on PII of Calves***

Whereas varying the level of iodine supplementation of dams in the precalving period did not affect the ability of the calves to absorb IgG, it did affect the levels of circulating iodine in the plasma of the calves. The high level of iodine fed to the cows in the HI treatment group was reflected in the greater concentration of iodine in the plasma of calves of both the HI\_HI and the HI\_MI treatment groups when sampled at 0 h of age. Circulating iodine in the pregnant cow is readily transported to the bovine fetus (Aschbacher et al., 1966; Miller et al., 1967; Guyot et al., 2011), and thus the high level of PII in the HI\_HI and the HI\_MI calves is not surprising.

Consistent with previous studies (Miller et al., 1967; Gilles et al., 2009), PII levels declined between 0 h and 24 h of age by 50, 50, 54, and 59% for calves in the MI\_MI, SI\_SI, HMI\_HMI, and MI\_HI treatment groups, respectively. Whereas the PII of calves in the HI\_HI and the HI\_MI treatment groups increased between 0 and 24 h of age, the difference between PII at 0 and 24 h was not significant for these groups, and the numerical increase could be a chance result attributable to the vagaries of the testing method.

In evaluating the iodine status of cattle, a PII between 106 and 285  $\mu\text{g/L}$  is considered adequate, and less than 50  $\mu\text{g/L}$  appears to be deficient (Mee and Rogers, 1996). Iodine deficiency in the pregnant cow or heifer can result in the birth of goitrous, weak, hairless, or dead calves (Hidiroglou, 1980; NRC, 2001). None of

the calves in the present study were born as such. It appears no PII levels have been defined as adequate for neonatal calves at present; however, considering the levels currently established for adult cattle, none of the calves were deemed deficient in iodine at either 0 or 24 h of age. In fact, at 0 h of age, all calves had a PII that exceeded the level defined as adequate. The PII at 0 h in calves of dams that received no additional supplementation was more than adequate, and therefore the results of the current study indicate that ensuring the diet of the precalving cow meet the minimum NRC requirements for the pregnant, nonlactating cow is sufficient to ensure the appropriate iodine status and health of the newborn calf, and that supplementing additional iodine is unnecessary for this purpose. However, the effects on the cow were not established as part of this present study, and this is an area that warrants further research.

### Practical Implications

Although the results of the current study suggest that over-supplementation of iodine in the precalving period does not affect the passive transfer of immunity to calves, we would urge producers not to interpret this as an encouragement to feed large quantities of iodine. Supplementation of the prepartum dairy cow with iodine above the level necessary to meet the minimum requirements established by the NRC was unnecessary to ensure appropriate iodine levels in calves at birth. In addition to being financially wasteful, few proven benefits exist to feeding more iodine than recommended, and iodine toxicity (which has been reported in adult dairy cows with dietary intakes of just 50 mg/d; NRC, 2001) can result in many deleterious outcomes. These include excessive nasal and ocular discharge, salivation, decreased milk production, coughing, and dry, scaly coats (Olson et al., 1984), an increase in reproductive disorders (Paulíková et al., 2002), and suppression of the immune system (Hillman and Curtis, 1980), possibly resulting in reduced ability to combat disease (Olson et al., 1984). The present study is one of only a limited number of studies carried out to investigate the effects of high iodine supplementation of the dairy cow on the IgG absorption in her calves, and further work in this area is warranted.

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