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Physical and physiological factors associated with twin- and triplet-born lamb heat production at 24 to 36 hours of age

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ABSTRACT

This study attempted to identify physical and physiological factors of twin- and triplet born lambs within the first 24 to 36 hours of life which are associated with maximum heat production on a per lamb basis (W/lamb) and a per kg of birth weight basis (W/kg). Multiple regression analyses revealed that lamb birth weight accounted for 34 percent of the variation in maximum heat production on a per lamb basis ($P < 0.001$), whereas, inclusion of plasma non-esterified fatty acids (NEFA; $P < 0.01$), tri-iodothyronine (T_3; $P < 0.05$) and glucose concentrations at 24 to 36 hours of age ($P < 0.05$) increased the accountability to 59 percent. Multiple regression analyses also revealed that plasma NEFA concentrations at 24 to 36 hours of age accounted for 15 percent of the variation in maximum heat production on a per kg of birth weight basis ($P < 0.01$), whereas, inclusion of plasma T_3 at 24 to 36 hours of age increased the accountability to 22 percent ($P < 0.05$). Overall these results suggest that having greater lamb birth weights, plasma thyroid hormone, glucose and NEFA concentrations at 24 to 36 hours of age is beneficial for twin- and triplet-born lamb thermoregulation during periods of cold stress.

Keywords: twin; triplet; birth weight; glucose; non-esterified fatty acids; tri-iodothyronine; heat production.

INTRODUCTION

Triplet-born lambs have been found to be lighter at birth and to have lower plasma thyroid hormone concentrations within the first 24 hours of life than twin- or single-born lambs (Stafford *et al.*, 2007). These factors suggest that triplet-born lambs have a greater capacity to lose heat and a reduced capacity to produce heat when compared to twin-born lambs. Observed lower rectal temperatures of triplet-born lambs at birth (Kerslake *et al.*, 2010b; Stafford *et al.*, 2007) and lower heat production on a per lamb basis (Kerslake *et al.*, 2010b; Kerslake *et al.*, 2010d) support this statement. Identification of physical and physiological factors associated with the lamb's ability to produce heat will provide useful information on which management factors should be targeted to improve the thermoregulation capability and survival of the triplet-born lamb. The aim of this study is to identify physical and physiological factors associated with maximum heat production on a per lamb basis (W/lamb) and per kg of birth weight basis (W/kg).

MATERIALS AND METHODS

This paper details an associative analysis of the physical and physiological factors influencing newborn lamb thermoregulation, using data from an experiment previously reported by Kerslake *et al.* (2010a). Full details of experimental design, management during gestation and measurements of physical and physiological factors and of maximum heat production are presented in Kerslake *et al.* (2010a).

Experimental design

Briefly, the experimental design of the study was a 2 x 2 x 2 factorial, which included two litter sizes (Twin and Triplet), two maternal iodine supplementation treatments (Supplemented and Non-supplemented) and two ewe nutritional treatments (Pasture and Kale). Thirty five days prior to mating half of a mob of 1,150 mixed-age Romney cross ewes were injected intramuscularly in the anterior half of the neck with 1.5 mL of iodised peanut oil (Flexidine®, Bomac Laboratories Ltd, Auckland). The other half of the mob was not injected. From mating until day 68 of pregnancy (P68), the ewes were grazed on *ad-libitum* ryegrass *(Lolium perenne)* and white clover *(Trifolium repens)* pasture. At P68 30 twin- and 30 triplet-bearing ewes were randomly selected from both the iodine supplemented and non-supplemented mobs. These groups were then further split with 14 twin- and 16 triplet-bearing ewes from the iodine supplemented and non-supplemented mobs being grazed on kale from P68 until P120, and then on *ad-libitum* ryegrass and white clover pasture until parturition. The remaining 16 twin- and 14 triplet-bearing ewes were grazed on *ad-libitum* ryegrass and white clover pasture from P68 until parturition.

Management during parturition

On P145, all ewes were supervised continuously for ten days. Ewes that showed signs of parturition were moved quietly to a 5 m by 3 m temporary pen located at the front of their paddock. The ewes remained in these pens during parturition with the ewes and lambs released from the pen 24 hours after the last lamb was born. Ewes that were penned gave birth with minimal disturbance, and each lamb was tagged and its birth rank recorded within 5 minutes of birth. Also at this time, two 5-mL blood samples were collected by jugular venepuncture (lithium heparin and potassium oxalate vacutainer, Becton Dickson Vacutainer Systems, USA) and the rectal temperature measured. The rectal temperature of each lamb was also recorded at 1, 3 and 12 hours of age, and another blood sample was taken at 3 and 24 hours of age (lithium heparin vacutainer, Becton Dickson Vacutainer Systems, USA). At 3 hours of age, the lamb was weighed and its sex determined. Ewes which gave birth in the paddock remained there, with no observations recorded and no measurements taken.

Measuring maximum heat production

At 24 to 36 hours of age, 18 twin (Supplemented, n = 2; Non-supplemented, n = 16) and 34 triplet (Supplemented, n = 17; Non-supplemented, n = 17) lambs born to ewes grazing kale followed by pasture, and 28 twin (Supplemented, n = 14; Non-supplemented, n = 14) and 30 triplet (Supplemented, n = 17; Non-supplemented, n = 13) lambs born to ewes grazing pasture only were randomly selected to determine their maximum heat production or summit metabolism, by indirect open-circuit calorimetry. Metabolic rate in Watts (W) and Watts per kg of birth weight was calculated as previously described by Kerslake *et al*. (2009).

Plasma analysis

Lamb plasma samples were analysed for glucose (hexokinase assay, Roche Diagnostics Ltd, Switzerland), fructose (Enzymatic assay, Biopharm, Darmstadt, Germany), lactate (lactate oxidase/peroxidase assay, Roche Diagnostics Ltd, Switzerland), thyroxine (T_4) and tri-iodothyronine (T_3) (Radioimmunoassay diagnostic kit, Coat-A-Count, Diagnostic Products Corporation, CA, USA), gamma-glutamyl-transferase (GGT) (Roche, Diagnostics Ltd, Mannheim, Germany), non-esterified fatty acids (NEFA) (Sigma, Illinois, USA), and Immuno-globulin (IgG) (Standard direct ELISA assay).

Statistical analysis

Univariate and multivariate analyses were conducted to identify which physical and physiological factors within 24 to 36 hours of age were associated with maximum heat production on a per lamb basis (W/lamb) and on a per kg of birth weight basis (W/kg).

Physical factors evaluated were birth weight and surface-area-to-birth-weight ratio. Physiological factors evaluated were plasma glucose, fructose, lactate, T_4, T_3 concentrations within five minutes of birth, plasma T_4, T_3 and creatine kinase concentrations at three hours of age, plasma GGT and IgG concentrations at 24 hours of age, plasma glucose, NEFA, T_4 and T_3 concentrations directly before calorimetry, and plasma glucose and NEFA concentrations directly after calorimetry.

A general linear model (PROC GLM; SAS, 2003) was first used to identify if maternal iodine supplementation, ewe nutrition, lamb birth rank and their potential interactions had any effect on lamb physical and physiological factors within the first 24 to 36 hours of life. To achieve a normal distribution, plasma NEFA concentrations directly before and after calorimetry were subjected to a $\log_{10}$ transformation and plasma GGT concentrations were subjected to a square root transformation before being analysed using a general linear models.

TABLE 1: Univariate analysis of the physical and physiological factors which had an affect ($P < 0.1$) on maximum heat production on a per lamb basis (W/lamb) at 24 to 36 hours of age. n = Number of lambs; SE = Standard error; T_3 =Tri-iodothyronine; T_4 = Thyroxine; NEFA = Non esterified fatty acids; IgG = Immunoglobulin G. The linear regression equations are of the form Y = Intercept + Slope x Variable.

Variable	n	Intercept	Slope ± SE	P value	R^2 (%)
Birth weight (kg)	110	-12.0	18.5 ± 2.31	<0.001	37.3
Glucose @ 24-36 hours of age (mmol/L)	104	34.2	5.6 ± 1.24	<0.001	16.8
T3 @ 24-36 hours of age (nmol/L)	103	31.7	7.1 ± 1.86	<0.001	12.6
T4 @ 24-36 hours of age (nmol/L)	104	35.6	0.2 ± 0.06	<0.001	11.8
NEFA @ 24-36 hours of age (mmol/L)	103	55.7	12.4 ± 3.60	<0.001	10.6
T4 @ 3 hours of age (nmol/L)	100	41.4	0.16 ± 0.05	0.005	8.0
T4 @ 5 minutes of birth (nmol/L)	101	46.1	0.15 ± 2.73	0.008	7.0
T3 @ 3 hours of age (nmol/L)	100	50.2	3.94 ± 1.66	0.02	5.5
IgG @ 24-36 hours of age (mg/mL)	104	60.5	0.16 ± 0.09	0.06	3.4
T3 @ 5 minutes of birth (nmol/L)	101	57.4	4.57 ± 2.46	0.07	3.4

TABLE 2: Multivariate analysis of the physical and physiological factors which had an affect on (P <0.05) maximum heat production on a per lamb basis (W/lamb) at 24 to 36 hours of age. n = number of lambs; SE = Standard error; T3 = Tri-iodothyronine; NEFA = Non esterified fatty acids. The multiple regression equations are of the form Y = -12.0 + 18.3 (birth weight) + 10.4 (NEFA before calorimetry) + 5.09 (T3 before calorimetry) + 2.66 (glucose before calorimetry).

Variable	n	Slope ± SE	P value	Partial R^2 (%)	Model R^2 (%)
Intercept		-12.0 ± 12.4	<0.001		
Birth weight (kg)	84	18.3 ± 2.36	<0.001	34	34
NEFA @ 24-36 hours of age (mmol/L)	84	10.4 ± 3.39	0.01	5	39
T3 @ 24-36 hours of age (nmol/L)	84	5.09 ± 2.08	0.02	4	43
Glucose @ 24-36 hours of age (mmol/L)	84	2.66 ± 1.27	0.03	16	59

TABLE 3: Univariate analysis of the physical and physiological factors which had an effect (P <0.1) on maximum heat production on a per kg of birth weight basis (W/kg) at 24 to 36 hours of age. n = number of lambs; SE = Standard error; T_3 =Tri-iodothyronine; T_4 = Thyroxine; NEFA = Non esterified fatty acids; IgG = Immunoglobulin G. The linear regression equations are of the form Y = Intercept + Slope x Variable.

Variable	n	Intercept	Slope ± SE	P value	R^2 (%)
NEFA @ 24-36 hours of age (mmol/L)	103	13.1	0.07 ± 0.59	<0.001	14.7
Glucose @ 24-36 hours of age (mmolL)	104	10.6	0.83 ± 0.21	<0.001	13.1
T_3 @ 24-36 hours of age (nmol/L)	103	10.4	1.00 ± 0.32	0.002	9.1
T_4 @ 24-36 hours of age (nmol/L)	104	12.0	0.03 ± 0.01	0.02	5.1
Surface-area-to-birth-weight ratio	110	21.2	-0.53 ± 0.02	0.04	3.8
IgG @ 24-36 hours of age (mg/L)	104	14.3	0.03 ± 0.01	0.08	3.0

A general linear model (PROC GLM; SAS, 2003) was used to identify any significant associations (P <0.1) between each individual physical and physiological variable and maximum heat production on a per lamb basis (W/lamb) and per kg of birth weight basis (W/kg). An ANCOVA was also used to identify any slope differences between lamb birth ranks for any significant associations found. All significant variables found in the univariate analysis were entered into a multiple linear regression model (PROC REG; SAS, 2003). A forward selection process was employed for the development of the model. If the variables were statistically significant at P <0.05 they remained in the model. The final model was checked for multicolinarity using the tolerance inflation factor.

RESULTS

Maternal iodine supplementation, ewe nutrition and their potential interactions had no (P >0.1) effect on any of the lamb physical and physiological measurements analysed in this analysis as reported by Kerslake *et al.* (2010a).

An increase in birth weight, plasma T_3 and T_4 concentrations within five minutes of birth and at three hours of age, plasma IgG, NEFA, T_4 and T_3 concentrations at 24 to 36 hours of age had a positive effect (P <0.1) on maximum heat production on a per lamb basis (Table 1). No slope differences (P >0.1) were found between twin- and triplet-born lambs for any of the significant associations found.

The final multiple regression analysis revealed that lamb birth weight accounted for 34 percent of the variation in maximum heat production on a per lamb basis, whereas, inclusion of plasma NEFA, T_3 and glucose concentrations before calorimetry increased the accountability to 59 percent (Table 2).

An increase in the surface-area-to-birth-weight ratio of the lamb had a negative effect (P <0.1) on maximum heat production on a per kg of birth weight basis (Table 3), where an increase in plasma IgG, glucose, NEFA, T_4 and T_3 concentrations before calorimetry had a positive effect (P <0.1) on maximum heat production on a per kg of birth weight basis. No slope differences (P >0.1) were found between twin- and triplet-born lambs for any significant associations found.

The final multiple regression analysis revealed that plasma NEFA concentrations before calorimetry accounted for 15 percent of the variation in maximum heat production on a per kg of birth weight basis, whereas, inclusion of plasma T_3 before calorimetry increased the accountability to 22 percent (Table 4).

TABLE 4: Multivariate analysis of the physical and physiological factors which affect (P <0.05) maximum heat production on a per kg of birth weight basis (W/kg) at 24 to 36 hours of age. n = number of lambs; SE = Standard error; T_3 = Tri-iodothyronine; NEFA = Non esterified fatty acids. The multiple regression equations are of the form Y = 11.6 + 1.89 (NEFA before calorimetry) + 0.63 (T_3 before calorimetry).

Variable	n	Slope ± SE	P value	Partial R^2 (%)	Model R^2 (%)
Intercept		11.6 ± 3.30	<0.001		
NEFA @ 24-36 hours of age (mmol/L)	101	1.89 ± 0.61	<0.001	14.8	14.8
T_3 @ 24-36 hours of age (nmol/L)	101	0.63 ± 0.34	0.005	6.6	21.5

DISCUSSION

This analysis attempted to identify physical and physiological factors within the first 24 to 36 hours of life which were associated with maximum heat production on a per lamb and per kg of birth weight basis.

Lamb birth weight was found to be positively associated with maximum heat production on a per lamb basis. The positive association between lamb birth weight and heat production (Kerslake *et al.*, 2009), the negative association between birth weight and surface-area-to-birth-weight ratio, and the positive association between surface-area-to-birth-weight ratio and heat loss are already well documented (McCutcheon *et al.*, 1983). Practical means of increasing lamb birth weights, and thus reducing the surface-area-to-birth-weight ratios should therefore have positive effects on the ability of the lamb to produce and conserve heat. Increasing lamb birth weights, through such practical means as improving maternal nutrition during pregnancy (Kerslake *et al.*, 2010c) and mid pregnancy shearing (Kenyon *et al.*, 2006), should improve the lamb's chances of survival during a cold stress event.

Physiological factors which were associated with maximum heat production on a per lamb basis include an increase in plasma glucose, NEFA and T_3 concentrations directly before the onset of cold stress. Plasma NEFA and T_3 concentrations were also associated with maximum heat production on a per kg of birth weight basis. Glucose and NEFA are both important energy sources for the production of heat (Eales & Small, 1981), where greater concentrations of plasma glucose have previously been identified as one of the most important determinants for high rates of heat production in the newborn lamb (Eales & Small, 1986). Plasma T_3 concentrations are also important for production of heat, where T_3 increases the expression and activation of UCP-1 (Symonds *et al.*, 2000), the proton channel found in brown adipose tissue which is responsible for heat production. In addition, associations between low plasma thyroid hormone concentrations and low rectal temperatures and increased susceptibility to hypothermia have previously been reported (Caple & Nugent, 1983). Having greater plasma concentrations of glucose, NEFA and T_3 directly before cold stress, should therefore have a positive effect on maximum heat production and consequently the ability of the lamb to thermoregulate during cold stress. Plasma GGT and/or plasma IgG concentrations at 24 to 36 hours of age, which were both measured as indicators of colostrum intake (Kenyon *et al.*, 2005), were found to be positively correlated with plasma glucose, NEFA and T_3 concentrations directly before cold stress (Kerslake, 2010). One potential practical way of increasing plasma T_3, glucose and NEFA concentrations may therefore be to ensure that the newborn lamb has been well fed and has access to a plentiful colostrum and/or milk supply. Previous experiments have shown that the intake of colostrum can increase the plasma concentrations of glucose and NEFA, which in turn can increase summit metabolism by 17-20% (Eales & Small, 1981). In addition, feeding 20-50 mL of milk to cold-exposed lambs can increase the plasma concentrations of T_3, colonic temperature and the thermogenic activity of brown adipose tissue (Symonds, 1995). The intake of colostrum is not only important for providing energy for heat production but also for developing a bond between the mother and the young (Nowak *et al.*, 1997) and sustaining teat-seeking behaviours (Alexander & Williams, 1966). Another potential practical approach to increase lamb plasma thyroid hormone concentrations are to ensure that maternal plasma concentrations of iodine and selenium are of a sufficient status. Maternal iodine and selenium deficiencies during late pregnancy have both been shown to decrease plasma T_4 or T_3 concentrations of the newborn (Caple & Nugent, 1983). Supplementing ewes with iodine and/or selenium in late pregnancy has shown mixed results however, with maternal iodine and/or selenium supplementation having both a positive (Rose *et al.*, 2007), a negative (Boland *et al.*, 2008) and non (Kerslake *et al.*, 2010d) effect on plasma T_4 and/or T_3 concentrations of the newborn lamb. Differences between studies may however be due to amount and route of delivery for iodine and selenium supplementation.

Overall these results suggest that greater lamb birth weights, plasma thyroid hormone, glucose and NEFA concentrations should help improve lamb thermoregulation during periods of cold stress. These physical and physiological factors however only accounted for 59 and 23 percent of maximum heat production on a per lamb basis and on a per kg of birth weight basis, respectively. So while practical means of improving the physical and physiological factors found in this study may offer some improvements in newborn lamb thermoregulation, further research is needed to explain the large amount of unaccounted variation in lamb heat production.

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