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BRIEF COMMUNICATION: Metabolic phenotypes associated with differential growth in twin foetuses in late gestation.

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Abstract
The objective of this study was to evaluate the relationship between foetal weight and targeted metabolomic profiles of twin foetuses in late gestation to provide insights into metabolic phenotypes associated with differential foetal growth. Archived foetal plasma samples from ewes managed in an outdoor forage-based production system were used for targeted metabolomic profiling where there was divergence in twin foetal weight at 135 days of gestation (4.0 to 6.5 kg; n=40). Pearson correlations between foetal weight and plasma metabolite profiles were determined. There were 7 positive and 4 negative correlations between metabolite profiles and foetal weight ($P<0.05$). Key metabolic phenotypes that differed between high and low foetal weight included nitric oxide and serotonin signalling, oxidative stress, and the supply of specific amino acids (arginine, lysine, histidine and threonine). These results provide insights into the metabolic pathways important for the regulation of foetal growth and development in late gestation in twin-born lambs.

Keywords: Sheep, metabolomics, oxidative stress, nitric oxide, serotonin, foetal growth and development.

Introduction

Improving lamb survival and growth is an important strategy to enhance animal welfare, productivity, profitability and ecoefficiency of sheep production in New Zealand pastoral systems (Mackay et al., 2019, Taylor et al., 2021, 2024). Lamb mortality is a multifactorial trait with multiple risk factors (Dwyer et al., 2016). It is well established that low birth weight is a key contributor to lamb mortality with low birth weight increasing the risk of starvation and exposure (McCoard et al., 2017). Increased knowledge of the metabolic regulation of foetal growth may provide insights into strategies to enhance birth weight and thus lamb survival.

Metabolomics is the evaluation of a set of metabolites in a biological sample that can represent the metabolic functioning of a cell, tissue or organism that reflect genetic, epigenetic and environmental factors that influence cellular physiology. Therefore, metabolomics is an important tool for exploring the physiological changes assocaited with foetal growth (Souza et al., 2019).

The objective of this study was to evaluate the relationship between foetal weight and targeted metabolomic profiles of twin foetuses in late gestation from ewes managed in an outdoor forage-based production system to provide insights into metabolic phenotypes associated with differential foetal growth in sheep.

Materials and methods

Samples used in this study were collected from a subset of animals in the study described in Hammond et al. (2022). Briefly, twin-bearing ewes grazed either a fodder beet (FB) or perennial ryegrass (RG) forage in situ with ad libitum ryegrass/clover hay. On day 135 of gestation, foetal blood samples were collected from 10 randomly selected twin-pairs from each treatment (FB and RG; total of 40 foetuses) via cardiac puncture, immediately prior to humane killing, using a syringe and transferred into potassium-EDTA containing vacutainers (Becton Dickinson Ltd., NZ). Foetal weight was recorded after humane killing and removal of exccess body fluids. Plasma was harvested and stored at -80^{o}C until targeted metabolic profiling using a commercial service (Absolute IDQ p180 kit, Biocrates Life Science, AG, Austria) according to the manufacturer's standard protocol as described by Kenez et al. (2018). This approach enabled evaluation of 188 metabolites from 6 compound classes (amino acids, biogenic amines, monosaccharides, acylcarnities, glycerophospholipids and sphingomyelins) and 136 metabolism indicators (MetaboIndicator™).

Pearson correlations between foetal weight and plasma metabolite profiles, independent of treatment, were determined using R version 4.4.1 (R Core Team, 2016).

Results and discussion

The average body weight was 4.90 kg (SD = 0.600) in the FB group and 5.30 kg (SD = 0.550) in the RG group. There were 7 positive and 4 negative correlations between metabolite/metabolic indicator profiles and foetal weight ($P<0.05$; Table 1).

Oxidative stress occurs when the production of reactive oxygen species (ROS) overwhelms intrinsic antioxidant defences (Burton and Jauniaux 2011). High levels of ROS can impair energy metabolism, cell signalling, cell cycle regulation and nutrient transport (Babel and Dandekar 2020; Keane et al., 2015), as well as impair immune and inflammatory reactions (Ling et al., 2021). Oxidative stress is commonly associated with foetal growth restriction in sheep (Sales et al., 2018) and can be caused by limiting the availability of active signalling molecules such as nitric oxide (NO; Gilbert et al., 2008) which is formed from arginine (Arg). Asymmetric

dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) are naturally occurring methylated metabolites of L-Arg that compete with L-Arg as a substrate for NO synthases leading to dysregulation of the NO metabolome (Grosse et al., 2020). The negative correlation between foetal weight and carnosine synthesis, NO synthase activity and ADMA and SDMA, coupled with the positive correlation with GABR and Arg highlight the importance of Arg bioavailability, abnormal ADMA and SDMA metabolism and oxidative stress. These results suggest dysregulation of the NO metabolome may be a key mechanism involved in the regulation of foetal weight in late gestation in sheep.

Table 1. Statistically significant Pearson correlations between foetal weight and metabolite and MetaboIndicator™ profiles in plasma of twin foetuses at 135 days of gestation.

Category	Metabolite	Pearson correlation	P-value
MetaboIndicator™	Carnosine synthesis	-0.417	0.007
MetaboIndicator™	NO synthase activity	-0.382	0.015
MetaboIndicator™	Sum ADMA + SDMA	-0.324	0.041
MetaboIndicator™	Assym. Arg. Methylation	-0.320	0.044
AA+Biogenic Amine	Histidine	0.312	0.050
AA+Biogenic Amine	Lysine	0.336	0.034
AA+Biogenic Amine	Serotonin	0.338	0.033
AA+Biogenic Amine	Threonine	0.343	0.030
Sphingomyelin	SM C20:2	0.382	0.015
MetaboIndicator™	GABR	0.382	0.015
AA+Biogenic Amine	Arginine	0.389	0.013

AA, Amino acid; ADMA, Asymmetric dimethylarginine; Assym. Arg., Asymmetrical Arginine; GABR, global arginine bioavailability ratio; SDMA, Symmetric dimethylarginine.

The positive correlation between Arg, lysine and histidine and foetal weight is consistent with the important role of specific amino acids in the regulation of foetal growth and development in sheep (McCoard et al., 2016). In addition, the positive correlation between circulating serotonin levels and foetal growth is consistent with the negative association between serotonin levels and oxidative stress, foetal hypoxia and stress and positive association with energy and amino acid metabolism and foetal growth (Domingues et al., 2023).

Collectively, the results of this study highlight the importance of oxidative stress, nitric oxide and serotonin signalling and supply of specific amino acids (notably arginine) in the regulation of foetal weight in late gestation twin-born lambs from ewes in forage-based production systems.

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