Veterinary Parasitology 236 (2017) 42–50

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

Can the amount of digestible undegraded protein offered to ewes during late pregnancy affect the performance and immune response of their offspring to gastrointestinal nematodes?

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ARTICLE INFO

Article history:
Received 22 December 2015
Received in revised form 17 January 2017
Accepted 21 January 2017

Keywords:
Faecal egg count
Digestible undegradable protein
Gastrointestinal nematodes
Lambs
Ewes
Performance

ABSTRACT

Maternal nutrition during pregnancy is a major environmental influence on foetal development with consequent effects on postnatal performance. We hypothesised that the level of intake of digestible undegraded protein (DUP) by the dam in late pregnancy would impact on the effectiveness of the immune response by offspring to gastrointestinal nematode infection. Eighty-five twin/triplet-bearing ewes, which were indoors from mid-pregnancy, were randomly assigned to 4 treatment groups for the final 6 weeks of pregnancy. Treatments were silage plus one of two iso-energetic and iso-nitrogenous concentrates (differing in DUP concentration; 29 and 94 g/kg DM) offered at one of two feed levels (18/30 and 24/35 kg in total for twin/triplet-bearing ewes, respectively). Ewes with triplets had one lamb removed at birth so that all ewes nursed 2 lambs when put to pasture as one flock in a 5-paddock rotational grazing system; all lambs were slaughtered after 29 weeks. Faecal egg count (FEC) and levels of serum IgA and IgE specific for *Teladorsagia circumcincta* were assessed for all lambs at various time points between 10 weeks of age and slaughter. Animal performance (live weight, live-weight gain, carcass weight) was recorded for all lambs. Worm burden at slaughter was determined for a sample of 12 lambs from each treatment. *Nematodirus* spp. FEC, 'other strongyles' FEC, and serum IgA and IgE specific for *T. circumcincta* were unaffected either by the concentration of DUP in the concentrate or by the level of concentrate offered to ewes in late pregnancy ($P > 0.1$). Likewise, the dietary regime of the dams had no effect on lamb performance ($P > 0.1$). It is concluded that increasing the DUP intake of ewes in late pregnancy had no effect on the immune response of their offspring to gastrointestinal nematode infection acquired through grazing naturally infected pasture.

1. Introduction

Gastrointestinal nematodes (GIN) are arguably the most pervasive problem facing sheep production worldwide, especially in young animals. The gastrointestinal tract of lambs is infected with various species of GIN; climatic conditions and age influence the predominant species present (Stear et al., 2009). In lowland sheep flocks, under Irish conditions, most lambs are born in March and flocks are managed on permanent pastures where lambs are first parasitized by *Nematodirus battus* (April/May); as the season progresses (May/June onwards) the main nematode challenge is from larvae of *Teladorsagia circumcincta* and *Trichostrongylus* spp. (Good et al., 2001, 2006). Traditionally, the control of parasitic nematodes in sheep has relied principally on a combination of grazing management and anthelmintic treatment (Miller and Horohov 2006; Patten et al., 2011). However, the widespread evidence for the development of anthelmintic resistance in sheep nematode populations (Kaplan, 2004; Papadopoulos, 2008; Good et al., 2012) has driven interest in alternative parasite control strategies (Sayers and Sweeney, 2005; Stear et al., 2007). One alternative strategy is to enhance the host's resistance by selective breeding (Miller and Horohov, 2006). However, substantial benefits from this approach are unlikely to be realized in the short term (Kahn et al., 2001) whereas a more immediate response may be possible through

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http://dx.doi.org/10.1016/j.vetpar.2017.01.021
0304-4017/

nutritional intervention to enhance the host's immune response to infection.

The effects of the composition of the diet offered to ewes during mid and late pregnancy on ewe performance, and on subsequent offspring performance, have been examined in various studies. A low level of nutrition during pregnancy has a negative impact on ewe body weight (BW) and body condition score (BCS) at lambing, and on lamb birth weight; all of which impact on subsequent lamb performance (Wallace, 1948; Khalaf et al., 1979; Keady and Hanrahan, 2010; Keady and Hanrahan, 2012a,b; Macarthur et al., 2014; Van Emon et al., 2014). Moreover, maternal nutrition can induce permanent changes in the structure, physiology and metabolism of the offspring (Wu et al., 2004; Symonds et al., 2006; Lan et al., 2013; Peñagaricano et al., 2014). For instance, Lan et al. (2013) evaluated the effect of maternal nutrition on a group of genes in foetal muscle and adipose tissue; the expression of 9 imprinted genes and 3 DNA methyltransferase genes were influenced by the maternal diet. In fact, the methylation levels of *CpG* islands of the genes *IGF2R* and *H19* were higher in foetuses from dams offered diets with a higher concentration of amino acids.

Previous studies have shown that host resistance to GIN is sensitive to metabolizable protein (MP) scarcity but not to moderate scarcity of metabolizable energy (ME) (Bown et al., 1991; Sakkas et al., 2009; Houdijk, 2012). While MP supplementation *per se* has been found to reduce the magnitude of faecal egg count (FEC) in growing lambs, protein quality may also be an important factor in the control of GIN (Houdijk, 2012). Thus, dietary inclusion of protein sources with a higher digestible undegraded protein (DUP): effective rumen degradable dietary protein (ERDP) ratio, such as fishmeal and xylose-treated soybean, generates a more efficient immune response to GIN compared to that observed when protein sources with lower DUP:ERDP ratios, such as cottonseed meal and sunflower meal, are used (Haile et al., 2004; Knox et al., 2006; Houdijk, 2012).

The net protein requirements for pregnancy (i.e., for gravid uterus + udder) double in twin-bearing ewes over the final 3 weeks of pregnancy due to accelerating foetal growth and mammary development (Robinson, 1983), and there are increasing reports on how the maternal diet during pregnancy can impact on 'foetal programming' (Lan et al., 2013; Wu et al., 2004). Protein scarcity may arise from low-quality forages poor in protein, inadequate feeding level (Coop and Kyriazakis, 1999; Houdijk, 2012) and/or parasitism (Coop and Kyriazakis, 1999). GIN may affect protein availability to the ewe either through a negative effect on voluntary food intake and/or a reduced efficiency of absorption of nutrients (Coop and Kyriazakis, 1999; Zaralis et al., 2009). In addition, endogenous protein losses due to leakage of plasma protein and the production of mucoprotein, as a consequence of nematode infection, must also be considered.

There is a paucity of information on the effects of the amount of DUP supplied to ewes in late pregnancy on the capacity of their lambs to resist parasitism. Hence, the aim of this study was to evaluate the effect of the level of DUP offered to ewes during the last 6 weeks of gestation on the development of immunity to GIN in their progeny, and on progeny growth. We hypothesised that different levels of DUP in the diet of pregnant ewes in late pregnancy would affect the development of resistance to GIN challenge in their offspring, with consequent effects on growth.

2. Materials and methods

2.1. Ethical approval

All procedures described in this study were conducted under experimental license from the Irish Department of Health in accordance with the Cruelty to Animals Act 1876 and the European Communities (Amendments of the Cruelty to Animals Act 1976 Regulations, 2002 and 2005).

2.2. Animals and experimental design

The lambs used in the study were born to the Belclare and Belclare × Scottish Blackface ewes described in Sebastiano et al. (2016), which received 1 of 4 dietary treatments during the final 6 weeks of pregnancy; the treatments were 2 concentrates differing in DUP concentration [low (L) and high (H)] that were each offered at 2 levels (L and H). The 2 concentrates were formulated to be iso-nitrogenous (206 g crude protein [CP] per 1 kg DM) and iso-energetic (12.5 MJ/kg DM) and to contain DUP concentrations of either 29 (L) or 94 (H) g/kg DM, respectively, using published values (AFRC, 1993) for crude protein (CP), ME and DUP for the individual ingredients. Twin-bearing twins offered the L and H concentrate feed levels were offered a total of 18 and 30 kg of concentrate supplement, respectively, while triplet-bearing ewes on the corresponding diets were offered 24 and 35 kg concentrate, respectively. Concentrate feeding management is described in Sebastiano et al. (2016). The Belclare × Scottish Blackface ewes had been mated by Suffolk rams, the majority of Belclare ewes had been mated by Belclare rams and the remainder by Suffolk rams; thus the lambs represented 3 genotypes. The lambs were born as twins or triplets, mean birth date 16 March, but litters were reduced to 2 before being put to pasture.

Within 3 days of birth, ewes and their lambs were turned out to pasture that had been grazed only by sheep in previous years, and were managed as one flock in a rotational-grazing system involving 5 paddocks. Sward height was recorded for each paddock, pre and post grazing, using a plate meter (Ashgrove Pastoral Products, Hamilton, New Zealand). The paddocks were managed to achieve the target sward heights described by Keady (2010). Lambs were weighed at birth and at weeks 5, 10, 14, 21 and 29 weeks of age; weaning was at 14 weeks. All lambs were dosed with a levamisole anthelmintic (Chanaverm; Chanelle, Loughrea, Co. Galway, Ireland) at 5 weeks of age to ameliorate negative consequences of *N. battus* challenge. They were treated, on 2 further occasions, with an oral ivermectin (Oramec; Merial Animal Health Ltd, Harlow, Essex, England); these treatments followed extended periods of nematode challenge (mainly from *T. circumcincta* and *Trichostrongylus* spp.) in order to maximise the opportunity for any dietary-treatment effects on immune competence to be revealed. The first of these treatments was predetermined to be administered at weaning (i.e., 14 weeks of age) while the timing of the second treatment was delayed until FEC (based on composite faecal samples) had reached a threshold of ≥2000 eggs per gram (epg) in at least one treatment group. All anthelmintic treatments were administered in accordance with the manufacturer's instructions.

All lambs were slaughtered, at week 29 of the study (7 October), in an abattoir approved by the European Union. Carcass weight, conformation and fatness were recorded for each lamb at slaughter; conformation and fat classification were assigned by abattoir staff on the basis of visual assessment according to the European Lamb Carcass Classification Scheme (DAFM, 2016). There were 5 conformation classes, E (=good), U, R, O, and P (=poor), which were coded as 5, 4, 3, 2, and 1, respectively, for data analysis, and 5 fat classes (1 = leanest to 5 = fattest).

2.3. Parasitological measurements

2.3.1. Herbage larval count

The number of infective third-stage (L3) larvae (classified as *Nematodirus* spp. or 'other strongyle' species) on herbage was deter-