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Association of polymorphisms in leptin and leptin receptor genes with circulating leptin concentrations, production and efficiency traits in sheep

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ABSTRACT

The relationships among nucleotide sequence polymorphisms in the genes for leptin (*LEP*) and leptin receptor (*LEPR*) and circulating concentrations of leptin and variables related to energy turnover were investigated in a Awassi-Merino crossbred sheep population. Blood sampled at several times during gestation and lactation was used for the assay of circulating leptin and for DNA extraction. Parts of the ovine *LEP* and *LEPR* genes were sequenced and, of a total of seven polymorphisms identified, two for each gene were used for genotyping. Feed intake and body weight were recorded for 199 ewes (age 2.9–9.4 years) daily and milk yield and composition were measured. The data were used for association studies between single nucleotide polymorphisms (SNPs), circulating leptin concentration and production traits. Both polymorphisms identified in the ovine *LEP* gene were associated with circulating leptin concentration ($P < 0.05$) and one SNP was associated with feed intake per unit milk production ($P < 0.05$). For the SNPs in the *LEPR* gene, there were significant effects for residual feed intake during lactation ($P < 0.05$) and for feed intake and residual feed intake at defined times during gestation ($P < 0.05$). We conclude that polymorphisms in the *LEP* and *LEPR* genes are associated with production and efficiency traits. The interaction between body condition, milk production, energy efficiency and leptin concentrations should be investigated in detail in future studies. Further studies are also needed to identify the specific roles played by *LEP* and *LEPR* in the regulation of circulating leptin concentrations and energy metabolism.

1. Introduction

Leptin is the product of the obese (*OB*) gene and is involved in the control of several important physiological functions including feed intake and energy expenditure. In cattle, there are reports of nucleotide sequence variants within the genes for leptin (*LEP*) and its receptor (*LEPR*), and associations with circulating leptin concentrations (Liefers et al., 2003, 2004, 2005; Nkrumah et al., 2005; Buchanan et al., 2007). Nucleotide sequence variants in *LEP* have also been shown to be associated with feed intake, carcass merit, body fatness, milk quantity and quality, and energy balance (Buchanan et al., 2002; Liefers et al., 2003; Schenkel et al., 2005; Giblin et al., 2010; Ekerljung et al., 2012; Li et al., 2013). However, the evidence is inconsistent—independent studies with cattle either failed to identify significant associations (Barendse et al., 2005) or reported effects that were not significant across all pregnancy and lactation (Liefers, 2004).

By contrast with cattle, there have been relatively few studies on leptin in sheep. Polymorphisms identified in exon 3 of the ovine *LEP* in Makoei/Makooei sheep in Iran (Hashemi et al., 2011) were found to be associated with body weight (Hajihosseinlo et al., 2012). Among three single nucleotide polymorphisms (SNPs) in coding regions of the ovine *LEP* gene, one was related to muscle growth in Suffolk lambs (Boucher et al., 2006). Another study using several sheep breeds (Romney, Merino, Coopworth, Corriedale, Poll Dorset,

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Suffolk) identified four polymorphisms in exon 3 of ovine *LEP*, three of which were non-synonymous (changing the amino acid) and were suggested to be associated with leptin concentration and function (Zhou et al., 2009). In a population of Aboureyhan sheep, two other polymorphisms were identified within intron 2 of ovine *LEP* and were found to be associated with fat-tail percentage and body and carcass weight (Barzehkar et al., 2009) (Supplementary Table S1). None of these studies reported on possible associations with circulating leptin concentrations. Moreover, quantitative trait loci (QTL) for leptin concentrations during various stages of the reproductive process did not align with the positions of ovine *LEP* on chromosome 4 or ovine *LEPR* on chromosome 1 (Jonas et al., 2011).

The present study was designed to identify nucleotide sequence variants in ovine *LEP* and *LEPR* in a population of crossbred Awassi-Merino sheep, and to test whether those variants are associated with circulating leptin concentrations, feed intake, body weight, feed efficiency (feed intake per unit milk production), residual feed intake or lactation performance.

2. Material and methods

2.1. Animals

We used animals from a resource population of crossbred Awassi-Merino sheep that had been developed to identify QTLs for a broad range of production traits in sheep (Raadsma et al., 2009b). Animals were housed and maintained in accordance with Australian guidelines and experiments were reviewed and approved by the University of Sydney Animal Ethics Committee. In brief, four Awassi (A) sires were mated to 30 Merino (M) ewes and four F_1 (AM) sires were backcrossed to Merino ewes. Backcross ewes (AMM) were again crossed with F_1 sires to generate double-backcross progeny (AM_AMM). In the experiment described here, 78, 62, 24 and 26 ewes were daughters of Sires 1 to 4, respectively. Females used for the leptin assay and genotyping were from the AMM (113 ewes) and AM_AMM (77 ewes) generations. Fifty-seven ewes were milked once daily in 2005, while milking frequencies were increased to twice for 99 ewes milked in 2006 and 34 ewes milked in 2007. Milk yields were recorded every second day, and milk components were analyzed weekly, as described previously (Raadsma et al., 2009a). The median value (5.6) was used for the lactose content since data were missing for many observations. One ewe was used in two experiments, but the observations were retained only from one experiment.

2.2. Feed intake and energy efficiency

The 190 crossbred animals used in the present study were kept in the same location under similar management in open housing (not on pasture) and fed using automatic feeders during the three years of the experiment (year 2005, 2006, 2007). The feeder software recorded date, time, animal identification, body weight (kg) and feed intake (g) during every entry by an animal into every feeder. These data were used to calculate daily feed intake and average body weight. Data from the feeders were quality checked to remove outliers and final datasets were prepared using the R software package, version 2.15.1 (R Development Core Team, 2008). Daily feed intake and body weight records were merged with data for lactation and leptin concentrations (by date and animal), and additional records on closest lambing date, lamb weight (from 83 ewes), body condition score (from 57 ewes), sire and genetic background (AMM and AM_AMM generations).

The stages of reproductive cycle were assigned by reference to lambing dates. A total of 15 stages were defined, six during gestation (1: day −78 to −51, 2: day −50 to −22, 3: day −21 to −11, 4: day −10 to −6, 5: day −5 to −2, 6: day −1), one at lambing (day 0) and eight during lactation (1: day 1, 2: day 2 to 5, 3: day 6 to 10, 4: day 11 to 15, 5: day 16 to 20, 6: day 21 to 50, 7: day 51 to 100, 8: day 101 to 179). The stages were defined on the basis of observed changes in production traits, with data combined for 1 to 5 day periods close to lambing, when changes were rapid, and for longer periods during other stages. Extremely early (day −353 to −287) days in pregnancy and late (day 572 to 1066) days in lactation were removed as a closer lambing date was probably missing in the database. Data were averaged over the defined 15 lactation stages for each ewe.

The feed offered to the ewes was 90% dry matter and contained 12.5 MJ per kg dry matter (values given by the feed producer). Lamb weight, if not available, was estimated as the average of the recorded weights (5 kg for single lamb; 8 kg for twins). Residual feed intake (RFI) and Feed Conversion Ratio (FCR) were used as indicators of energy balance and efficiency. The following equations were used:

$$\text{Energy from feed intake [MJ]} = \left(\left(\frac{\text{daily feed intake[g]}}{1000 \times 0.9}\right) \times 12.5\right)$$

$$\text{Energy for maintenance [MJ]} = \text{body weight}^{0.75} \times 0.26$$

$$\text{Energy for milk production [MJ]} = \left(36.8 \times \left(\frac{\text{fat content [\%]}}{100}\right) \times \frac{\text{milk yield [L]}}{1000 \times 1.04}\right) + \left(17.9 \times \left(\frac{\text{protein content [\%]}}{100}\right) \times \frac{\text{milk yield [\%]}}{1000 \times 1.04}\right) + \left(16 \times \left(\frac{\text{lactose content [\%]}}{100}\right) \times \frac{\text{milk yield [\%]}}{1000 \times 1.04}\right)$$

$$\text{Energy for pregnancy [MJ]} = \frac{(36.9644 \times (\exp(-11.465)) \times (\exp(-0.00643 \times t)) - 0.00643 - t) \times \left(\frac{\text{lamb birth weight}}{4}\right)}{0.13}$$

where t is the time of gestation (average of the defined days for physiological stages).

Expected feed intake needed was calculated as:

$$\text{Energy use during gestation[MJ]} = (\text{Energy for maintenance[MJ]} + \text{Energy for pregnancy[MJ]})$$

$$\text{Energy use during lactation[MJ]} = (\text{Energy for maintenance[MJ]} + \text{Energy for milk production[MJ]})$$

$$\text{Residual feed intake} = (\text{Energy from feedintake[MJ]} - \text{Expected feed intake needed[MJ]})$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{feed intake [kg]}}{\text{milk yield [L]}}$$

2.3. Circulating concentrations of leptin

Blood was sampled three to nine times per animal by jugular vein puncture at various times during gestation and lactation in 2005 (56 animals), 2006 (92 animals) and 2007 (30 animals). Plasma samples were assayed for leptin with a double-antibody radioimmunoassay using an antibody raised against recombinant