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SINGLE NUCLEOTIDE POLYMORPHISMS IN THE BOVINE LEPTIN GENE AND THEIR ASSOCIATION WITH CARCASS AND EFFICIENCY TRAITS, AND ENDOCRINE PROFILES, IN FEMALE ANGUS COWS

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SUMMARY

One hundred and fifty female Angus cattle were genotyped for the bovine leptin gene SNPs UASMS1, UASMS2, E2FB and E2JW. Net Feed Intake (NFI) Estimated Breeding Values (EBVs) and E2JW SNP data was also acquired from 169 Angus cattle that originated from Trangie Research Station, NSW, and were selected for a divergence in feed efficiency. The E2JW SNP was associated with NFI, NFI EBV and P8 fatness. The UASMS1 and UASMS2 SNPs were associated with circulating leptin concentrations. These particular associations have not been reported previously but similar associations have reported in North American studies. The inconsistent associations suggest that these SNPs are not good candidates for marker-assisted selection for NFI. Also, the investigation of associations with endocrine profiles that reflect body composition such as leptin, requires genotyping of a larger number of Australian cattle than was possible in this experiment.

INTRODUCTION

Marker-assisted selection (MAS) for economically important traits in cattle has the potential significantly to alter the rate of genetic improvement, particularly when the marker-trait association is strong. Several studies over the past few years have explored the association between single nucleotide polymorphisms (SNPs) in an exon and the promoter region of the bovine leptin gene and various carcass, growth and production traits (Buchanan *et al*. 2002; Kononoff *et al*. 2005; Nkrumah *et al*. 2005; Schenkel *et al*. 2005). The leptin gene was chosen as a focus of research because leptin has a role as a lipostatic signal that regulates whole-body energy metabolism. Leptin is synthesised by white adipocytes (Zhang *et al*. 1994) and has a role in the regulation of appetite, reproductive performance and food intake . It also affects body composition (Schenkel *et al*, 2005). This makes leptin one of the best physiological candidate markers for liveweight, feed intake, energy expenditure, reproduction and certain immune system functions. Relationships between leptin SNPs and fatness, lean meat yield, eye muscle area, marbling, growth, ultrasound back fatness, feed intake, NFI and serum leptin concentrations have been established but their associations with these traits have not been consistently verified across studies (Schenkel *et al*. 2005). Most of the studies have been undertaken on North American cattle populations (Buchanan *et al*. 2002; Nkrumah *et al*. 2005; Schenkel *et al*. 2005) and they all reached similar conclusions about the associations between SNP and carcass, growth and production traits. However, when Barendse *et al*. (2005) investigated a SNP in a large population of Australian cattle, they concluded that marker-trait associations that exist in North American cattle populations may not exist in Australian cattle populations. Only one of the North American studies included female cattle in the analysis (Schenkel *et al*. 2005) and thus little information exists about marker-trait associations in breeding cattle. Identification of strong SNP/trait

associations in Australian cattle and their relationships to carcass and efficiency traits has the potential considerably to enhance the ability of producers to select for desirable and economically beneficial, heritable traits in their cattle.

AIMS AND HYPOTHESES

The aims of this experiment were to identify associations between the SNPs and carcass traits or feed efficiency; to identify associations between the SNPs and pre- and post-calving endocrine profiles; and to use NFI EBV data to validate the results for SNP/trait association for the E2JW SNP. It was hypothesised that there would be associations between the SNPs and carcass and efficiency traits. These would be reflected in SNP associations with endocrine profiles, particularly leptin, which would be an indirect measure of fatness. Also, there would be no association between SNPs and feed efficiency to suggest that a SNP could be used in MAS for NFI.

MATERIALS AND METHODS

Blood samples were collected from 150 female Angus cattle at Vasse Research Centre, Busselton, Western Australia (VRC animals). These animals were part of a larger, Cooperative Research Centre-funded experiment, namely the Maternal Productivity project. The animals were genotyped for the SNPs UASMS1 and UASMS2 in the promoter region and E2FB and E2JW in the exon region of the bovine leptin gene. Additionally, data were acquired for 169 Angus cattle, both male and female, originating from the Trangie Research Station, NFI-selected, herd. This expended data set (EDS) was used to increase the number of cattle genotyped for the E2JW SNP. Mid-parent NFI EBV data were also acquired for these animals. DNA extraction and SNP analysis was done by Saturn Biotechnology, Murdoch University and Biosciences Research Division, Department of Primary Industries, Victoria.

STATISTICAL ANALYSES

Linear mixed models were used to examine the relationships between leptin SNPs and the carcass and efficiency variables and the endocrine variables. All models included date of birth as a covariate since animals had different birth dates within the same year. Models for endocrine variables also included the effect of experimental treatments. All analyses were carried out using GenStat 11th edition (VSN International Ltd, Hertfordshire, UK)

RESULTS

Table 1. Statistical significance (P-values) of SNP effects on carcass (P8, EMA and IMF) and efficiency (NFI) traits and endocrine profiles in VRC animals (ns = non-significant i.e. P>0.05)

SNP		UASMS1	UASMS2	E2FB	E2JW
NFI		0.422	0.100	0.065	**0.005**
P8		0.953	0.132	0.076	**0.050**
IMF		0.941	0.560	0.371	0.719
EMA		0.565	0.409	0.432	0.991
Leptin	Pre-calving	**<0.001**	0.873	**<0.001**	0.758
	Post-calving	**<0.001**	0.526	**<0.001**	0.840
IGF1	Pre- and post-calving	ns	ns	ns	ns
GH	Pre- and post-calving	ns	ns	ns	ns
Insulin	Pre- and post-calving	ns	ns	ns	ns

The associations between E2JW SNP and NFI and P8 were significant (Table 1). Figure 1 shows that the AA genotype in the E2JW SNP had significantly lower NFI than the AT or TT genotypes. A similar pattern exists for P8 whereby homozygous AA animals have a significantly lower ultrasound P8 measure than heterozygotes or homozygous TT animals.

Animals carrying the T allele for UASMS1 SNP and animals carrying the C allele for E2FB SNP had significantly higher mean pre- and post-calving leptin concentrations than the heterozygotes. Pre-calving leptin concentrations were uniformly higher than post-calving. There was no significant association among any of the SNPs and ultrasound carcass measures pre- or post-calving. There were no other associations between pre- and post-calving endocrine profiles and SNPs.

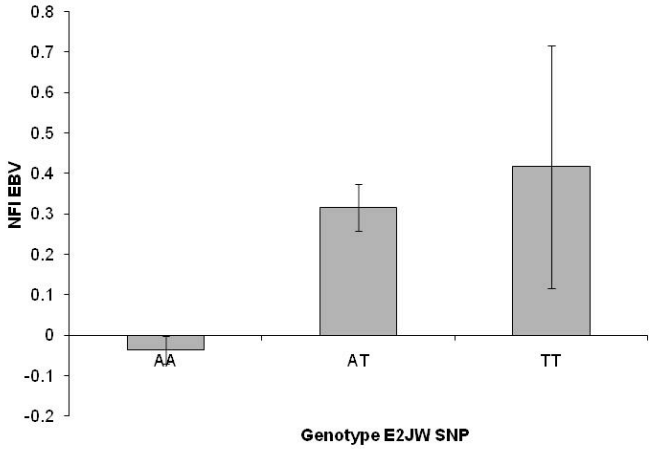

Figure 1. Mean NFI (determined by feed test) for AA, AT and TT genotypes of the E2JW SNP in the VRC animals. Error bars represent standard errors.

Table 2 shows the count and mean NFI EBV for the AA, AT and TT genotypes of the E2JW SNP in the EDS. The homozygous AA animals had a significantly lower mean NFI EBV than the heterozygotes or the homozygous TT animals

Table 2. Count and mean NFI EBV for the AA, AT and TT genotypes of the E2JW SNP in the EDS

	E2JW SNP			
Genotype	AA	AT	TT	F pr.
Count	221	81	3	
NFI EBV	-0.036	0.315	0.417	**<0.001**

DISCUSSION

There were few SNP/trait associations identified in the Australian experimental population used in this work, whereas North American studies reported numerous SNP/trait associations between SNPs and fatness, lean meat yield, EMA, marbling, growth, ultrasound measures of back fat, feed intake and NFI (Buchanan *et al*. 2002; Kononoff *et al*. 2005; Nkrumah *et al*. 2005; Schenkel *et al*. 2005). Moreover, for the E2JW SNP, animals with an A allele had significantly

less P8 fat measured on ultrasound, whereas Schenkel *et al*. (2005) reported the opposite, viz that the it was the T allele that was associated with higher lean meat yields and lower measures of fatness. E2JW SNP/trait associations have not previously been examined in Australian cattle. The absence of any other SNP/trait association concurs with the findings of Barendse *et al*.(2005), who examined similar SNP/fatness trait associations for the E2FB SNP in a large number (3129) of cattle and found no association with several fatness traits. It is not possible to draw firm conclusions pertaining to associations between SNP and carcass traits from the current experiment because of the small number of animals used, however, the results generally support the conclusion reached by Barendse *et al*.(2005) that the leptin SNPs are unlikely to be of genetic importance in Australian cattle.

The association between the E2JW SNP and NFI EBV (Table 2) identified in the EDS whereby animals with the T allele had higher (less favourable) NFI EBVs, has not been reported before. Where others (Nkrumah *et al*. 2005) have reported associations between SNP and NFI, in particular for the UASMS2 and E2FB SNP, no such associations were found in the results from this experiment. However, the pattern of homozygous AA animals recording lower NFI values than heterozygotes or homozygous TT animals was the same in this experiment. The results from this experiment identify the potential to associate leptin gene SNPs with feed efficiency which would assist MAS, but the results are not consistent with those other studies and need to be validated across a larger population, of particularly Australian cattle, of varying ages and sex.

Given the importance of energy balance on the efficiency and productivity of a beef herd, it was useful to investigate the association between leptin gene SNPs and various indicators or regulators of physiology, in particular fat distribution and metabolism. Similar to the results in this experiment, Nkrumah *et al*. (2005) reported that the T allele of UASMS2 was significantly associated with serum leptin concentrations ($P < 0.001$). Buchanan *et al*. (2002) found that when analysing the E2FB SNP animals with the T allele expressed higher levels of leptin mRNA than those with the A allele, a result similar to the one in the current experiment. The only significant relationships in this experiment were between the UASMS1 and E2FB SNP and pre- and post-calving leptin. It has been shown that serum leptin is positively associated with liveweight and body fatness (Chilliard *et al*. 1998) but unlike Nkrumah *et al*. (2005), the absence of an association with fatness in this experiment suggests that these SNPs do not represent functional mutations. The results of the current experiment suggest that identifying leptin gene SNP in Australian cattle is unlikely to be a useful tool in the development of MAS, particularly when considering the desirable heritable traits NFI and leanness. Although there were some SNP/trait associations with carcass traits, they were not the same as those previously reported and probably of little industry relevance.

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