Improving Stress Coping Ability: Comparison Between The *CYP17* Genotype Of *Ovis Aries* And *Capra Hircus*

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Introduction

In mammals, physiological stress stimulates the release of glucocorticoids (cortisol and corticosterone) from the adrenal cortex that results in glucose production at the expense of glycolysis (Munch (1971)). Cytochrome P450 17α-hydroxylase/17,20-lyase (CYP17) plays a critical role in the production of these glucocorticoids. Abnormalities in the *CYP17* gene or protein can result in various mild to lethal disorders (Payne and Hales (2004)). In the case of the South African Angora goat (*Capra hircus*), the activities of the two CYP17 isoforms was found to be the main cause for hypocortisolism that results in an increased susceptibility to cold stress (Engelbrecht et al., (2000); Storbeck et al. (2007)). In addition, Engelbrecht et al. (2000) showed that the stimulation of the hypothalmo-pituitary-adrenal (HPA) axis with insulin challenge *in vivo* resulted in less cortisol being produced in Angora goats when compared to South African Boer goats (*Capra hircus*) and Merino sheep (*Ovis aries*).

Two *CYP17* genetic sequences have been identified for Angora goats, namely ACS- (GenBank accession no. EF524063) and *CYP17* ACS+ (GenBank accession no. EF524064) that is 100% homologous with Boer goat *CYP17* (GenBank accession no. AF251387) (Storbeck et al. (2007)). These sequences differ with four single nucleotide polymorphisms. In Merino sheep, two genetic sequences have been identified, namely *CYP17* WT1 (GenBank accession no. L40335) and WT2 (GenBank accession no. AF251388) (Storbeck et al. (2007)) that differ with two single nucleotide polymorphisms.

This study investigates the importance of the *CYP17* genotype in cortisol production in the Merino sheep and Angora goat adrenal gland as potential genetic marker for stress coping ability in selection programs to ultimately improve livestock fitness.

Material and methods

Animals. Merino sheep from a breeding program were used, where divergent selection was based on maternal ranking values for number of lambs weaned per mating. At present, both reproduction (Cloete et al. (2004)) and stress coping ability, in terms of cortisol production in response to insulin challenge (Van der Walt et al. (2009)), is higher in the positive selected

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(H-line) than the negative selected line (L-line). South African Angora, respectively, were randomly selected from the same flock.

***CYP17* genotyping with real-time polymerase chain reaction (PCR).** Blood samples were collected from the jugular vein of adult sheep and goats, or from the heart chamber of dead lambs (2008 lambing season). The DNA isolation kit for mammalian blood (Roche, Germany) was used to isolate genomic DNA, which was then genotyped using the real-time PCR method developed by Storbeck et al. (2008).

***CYP17* copy number determination.** Relative copy number determinations were performed on the three goat genotypes (H_o, H_u and H_e) and heterozygous (WT1/WT2) sheep using quantitative PCR (Storbeck et al. (2008)). Fold change values were calculated relative to an H_o genotype calibrator using the $\Delta\Delta C_t$ method (Livak and Schmittgen (2001)).

Stress test. Merino sheep in the above mentioned breeding program (17 H-line and 21 L-line rams, 2-6 years of age) and a group of 30 Angora goats (3 groups of 10 goats for each *CYP17* genotype: 5 ewes and 5 rams, 14 months of age) were injected intravenously with human insulin at a dose of 0.1 IU/kg body mass after which blood samples were collected at times: 0, 15, 30, 60, 90 and 120 min. Blood plasma glucose and free cortisol was determined with RIA. Ethics approval was obtained from the Departmental Ethics Committee for Research on Animals (DECRA ref: R08/21).

Statistical analyses. The Chi-square test was used to analyze *CYP17* genotype frequencies. For the stress test, the total HPA axis response to insulin induced hypoglycemia was calculated from the area under the curve that represents the amount of plasma cortisol produced (nmol/L) per plasma glucose drop (mmol/L) at times 0, 15, 30, 60, 90 and 120 min post insulin challenge. ANOVA tests were done for these responses, as well as for the relative DNA copy number determinations, followed by Bonferonni's post-test. Graphs represent the mean and standard error of the mean. GraphPad Prism (version 4) software (GraphPad Software, US) was used for all analysis with α=0.05 for statistical significance.

Results and discussion

Merino *CYP17* genotyping. The real-time PCR method developed by Storbeck et al. (2008) to genotype Angora *CYP17* (ACS- and ACS+), was also suitable to genotype the two Merino sheep *CYP17* sequences: WT1 and WT2. The *CYP17* frequency distribution of 144 adult Merino sheep (112 H-line and 32 L-line) in the breeding program is 86.1% heterozygous WT1/WT2 and 13.9% homozygous WT1/WT1. No significant association (P=0.6722, Chi-square=0.7944, df=2) was found between *CYP17* genotypes and designation of population sample (H-line, L-line or lamb mortalities). Similar *CYP17* frequencies were observed in the case of Angora goats (Storbeck et al. (2008)). Interestingly, the homozygous WT2/WT2 genotype was not detected in Merino sheep, while in the case of Angora goats, the homozygous ACS+/ACS+ genotype was also not detected (Storbeck et al. (2008)).

Relative *CYP17* copy number determination. The absence of a homozygous genotype in both Angora and Merino suggests that either the homozygous genotype is lethal, or that there

is an underlying genetic aberration for the *CYP17* gene. Storbeck et al. (2008) did a relative *CYP17* copy number determination for Angora goats with Boer goats and Merino sheep as controls. Their data revealed that three *CYP17* genotypes exist in the case of the Angora goat, namely H_o, H_u and H_e. The H_o genotype has only one *CYP17* gene, namely ACS-. In contrast, the H_e genotype has two *CYP17* genes, where the ACS- locus is always present together with the ACS+ locus. This is the reason why the homozygote for ACS+ is never detected. Crossing H_o and H_e goats would yield the proposed intermediate H_u genotype. This genotype would receive both ACS- and ACS+ loci from the H_e parent, but only the ACS- locus from the H_o parent. The ACS- : ACS+ ratio in the H_u genotype would therefore be 2:1.

Storbeck et al. (2008) also confirmed that there is only one *CYP17* gene in sheep with two alleles: WT1 and WT2. Given this data, three genotypes would be expected in the population: homozygous WT1/WT1; heterozygous WT1/WT2 and homozygous WT2/WT2. However, no homozygous WT2/WT2 genotype was detected (n=180). The possibility that this genotype is lethal is therefore considered.

The *CYP17* genotype was thus determined for lambs that died during the peri-parturition period. However, the WT2/WT2 genotype remained undetected. It is suggested that the earlier stages in fetal or embryonic development be tested for the WT2/WT2 *CYP17* genotype. In addition, the *CYP17* genotyping test should be optimized for Merino sheep *CYP17* detection, since it was originally designed for goat *CYP17* genotyping.

Angora goat stress test. The total response of Angora goats to insulin challenge is depicted in Figure 1A. One-way ANOVA indicated that the *CYP17* genotype affected the ability of Angora goats to respond to insulin-induced hypoglycemia. The Bonferonni's post-test showed a reduced response in the H_o group compared to the H_u and H_e groups. No significant difference in total response was observed between the latter groups. These results suggest that the presence of *CYP17* ACS+ in the H_e and H_u genotypes is more advantageous for cortisol production than the presence of *CYP17* ACS-.

Merino sheep stress test. When the Merino sheep responses to insulin challenge were grouped according to *CYP17* genotype, the *CYP17* WT1/WT1 group had an improved response compared to the *CYP17* WT1/WT2 group (data not shown). Interesting results were obtained where H- and L-line Merino sheep in the breeding program were subdivided into *CYP17* genotypes (Fig. 1B). Two-way ANOVA indicated that the interaction between selection line and *CYP17* genotype is significant. Bonferonni's post-test revealed that the response was the same for all groups, except for L-line heterozygous WT1/WT2 sheep where a lower response was observed. These results indicate that the presence of *CYP17* WT1 is more advantageous for cortisol production than the presence of *CYP17* WT2 in the L-line where there is arguable an accumulation of low fitness alleles. This finding suggests that the effect of *CYP17* genotype is dependent on the interaction with other factors along the HPA axis. *CYP17* can thus be used as a genetic marker (selection for WT1) to improve fitness in selection lines with an inherent low ability to cope with stress.

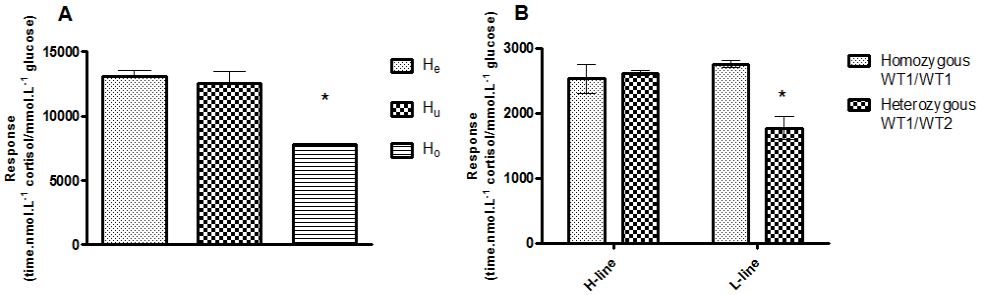

Figure 1: The total cortisol response to glucose decrease of (A) Angora goats and (B) Merino sheep (H- and L-line) grouped according CYP17 genotype.

Conclusion

The *CYP17* genotype is a factor that exerts its effect on stress coping ability via cortisol production in the adrenal cortex. This effect of the *CYP17* genotype seems to be dependent on the fitness of the sheep, or more specifically, the accumulation of other traits that also affect cortisol production. Furthermore, genetic variation in *CYP17* exists among species which results in inherently different abilities to produce cortisol. The unique genotypes in the South African Angora goat differ not only in terms of the genetic sequences encoding for *CYP17*, but also in copy number. The genotypes in the Merino sheep, however, differ only in terms of the genetic sequences encoding the one *CYP17* gene. It is suggested that the missing WT2/WT2 genotype in sheep may be lethal or that the *CYP17* genotyping test needs to be optimised for Merino sheep. This study showed that the *CYP17* genotype is a relevant genetic marker to include in selection criteria to improve stress coping ability of livestock. Further research is needed for the better understanding of the impact of *CYP17* genotypes on stress in livestock.

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