ARTHROSPIRA PLATENSIS: A NOVEL FEED SUPPLEMENT INFLUENCES GENE EXPRESSION IN THE HEART, KIDNEY AND LIVER OF PRIME LAMBS

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SUMMARY

Transcriptional patterns in the expression of genes controlling lipid metabolism in supplemented sheep are currently unknown, thus quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was used to assess the messenger RNA (mRNA) transcription levels and expressions of Aralkylamine N-acetyltransferase (*AANAT*), Adrenergic beta-3 receptor (*ADRB3*), B-cell translocation gene 2 (*BTG2*), and Fatty acid synthase (*FASN*) genes. The aim was to assess the effect of dietary supplementation with *Spirulina*, a blue-green cyanobacterial microalga, on the expression of genes controlling fatty acid metabolism in the heart, kidney and liver of prime lambs. In total, 432 heart, kidney and liver samples from 48 purebred and crossbred Merino prime lambs supplemented with low or high levels of *Spirulina* over a 9-week period were utilized for the study. Both the low and high levels of *Spirulina* supplementation regimes strongly up-regulated the transcription of all the selected genes. Sire breed and sex of lamb did not influence gene expression patterns; however, significant variations in response to *Spirulina* supplementation underpin the genetics-nutrition interactions that could be of practical importance for manipulating meat quality in the Australian dual-purpose prime lamb industry for a healthy polyunsaturated fatty acid profile.

INTRODUCTION

Spirulina (*Arthrospira platensis*) is a blue-green cyanobacterial microalga that contains 60-70% protein, high levels of carotenoids, essential vitamins, minerals and fatty acids (Ciferri, 1983, Holman and Malau-Aduli 2013; Holman *et al*. 2014). *Spirulina* has been trialled as a novel supplementary feed in many animal species and its recent inclusion in sheep diets has proven to be an effective nutritional strategy for increasing sheep meat production (Holman *et al*. 2012; Holman and Malau-Aduli, 2013). To our knowledge, apart from Kashani *et al*. (2015a) and Malau-Aduli and Kashani (2015), there is no available information on gene expression and molecular genetics-nutrition interactions between ovine organs and dietary supplementation with *Spirulina* in dual-purpose sheep. Therefore, the aim of this study was to investigate changes in the mRNA expression and transcriptional patterns of the following genes controlling lipid metabolism in the heart, kidney and liver of sheep under various *Spirulina* supplementation regimes: Aralkylamine N-acetyltransferase (*AANAT*), Adrenergic beta-3 receptor (*ADRB3*), B-cell translocation gene 2 (*BTG2*), and Fatty acid synthase (*FASN*).

MATERIALS AND METHODS

RNA was extracted from 432 heart, kidney and liver samples from 48 prime lambs sired by White Suffolk, Black Suffolk, Dorset and Merino rams randomly allocated to 3 treatment groups: the Control, grazing without *Spirulina* (0%), low (100mL/head/day in the ratio of 1g of *Spirulina* powder:10mL of water or 10% wt/vol), and high (200mL/head/day in the ratio of 2g of *Spirulina* powder:10mL of water or 20% wt/vol) *Spirulina* supplementation levels. The supplementary feeding trial continued for nine weeks after an initial three weeks of adjustment. The RNA samples were treated with PureLink^{TM}DNase (Life Technologies Pty Ltd. VIC, Australia), purified using RNeasy Mini Kit (Qiagen Ltd., VIC, Australia), DNase-treated and reverse transcribed to cDNA

using cDNA Synthesis Kit (Bioline Pty Ltd. NSW, Australia) and the primers in Table 1. Ubiquitin C (*UBC*) and Peptidyl-prolyl cis-trans isomerase A (*PPIA*) were used as house-keeping genes to normalise the gene expression data. Quantitative real time PCR *(qRT-PCR)* was carried out in triplicate 20 µL reactions containing 4 µL cDNA (50 ng), 10 µL 2×SensiFAST SYBR No-ROX Mix (Bioline Pty Ltd., NSW, Australia), 4.4 µL H_2O, and 0.8 µL forward and reverse primers (100 fmol). Assays were performed using the following cycling parameters: 95°C for 2 min (polymerase activation); 40 cycles of 95°C for 5 s (denaturation), 60°C for 10 s (annealing), and 72°C for 5 s (extension). Gene expression levels were recorded as cycle threshold (Ct) values, i.e. the number of PCR cycles at which the fluorescence signal is detected above the threshold value. Amplification efficiencies were determined for all candidate and reference genes using the formula E=10^(−1/slope), with the slope of the linear curve of cycle threshold (Ct) values plotted against the log dilution (Higuchi *et al*., 1993). The software package Rotor-Gene 3000 version 6.0.16 (Qiagen Pty Ltd., VIC, Australia) was used for efficiency correction of the raw Ct values, inter-plate calibration, normalisation to the reference gene, calculation of quantities relative to the highest Ct, and $\log_2$ transformation of the expression values for all genes. The qRT-PCR results were calibrated and normalized using the qBase relative quantification software (Pfaffl, 2001). A generalised linear model (GLM) in SAS (2009) was used in computing the fixed effects of *Spirulina* supplementation level, tissue, sire breed and sex, and their interactions on mRNA expression. Bonferroni's probability pairwise comparison test was used to separate mean differences, with the level of significance defined as $P<0.05$.

Table 1. Quantitative real-time PCR (qRT-PCR) oligonucleotide primers.

[a]Gene symbol	qPCR Primers		[b]T_a	Size (bp)
	Forward Primer	Reverse Primer		
AANAT	ACTGACCTTCACGGAGATGC	TTCACTCATTCTCCCCGTTC	60	211
ADRB3	TCAGTAGGAAGCGGGTCGGG	GGCTGGGGAAGGGCAGAGTT	60	291
BTG2	CTGGAGGAGAACTGGCTGTC	AAAACAATGCCCAAGGTCTG	60	194
FASN	GTGTGGTACAGCCCCTCAAG	ACGCACCTGAATGACCACTT	60	110
UBC	CGTCTTAGGGGTGGCTGTTA	AAATTGGGGTAAATGGCTAGA	60	90
PPIA	TCATTTGCACTGCCAAGACTG	TCATGCCCTCTTTCACTTTGC	60	72

[a]Aralkylamine N-acetyltransferase=*AANAT*, *β*3-adrenergic receptor=*ADRB3*, B-cell translocation gene 2= *BTG*2, Fatty acid synthase=*FASN*, Ubiquitin C=*UBC*, Peptidyl-prolyl cis-trans-isomeraseA=*PPIA*,
[b]T_a= Annealing Temp

RESULTS AND DISCUSSION

The relative mRNA expression levels of *AANAT*, *ADRB3*, *BTG2* and *FASN* genes in the heart are presented in Figure 1. Significant up-regulation of the *FASN* gene ($P<0.041$) in the heart, corresponding to a 21-fold change was observed in the low group. *AANAT* gene transcription was 4-fold and *BTG2* 1.4-fold up-regulated in the high treatment.

Kidney expression levels of *AANAT* and *ADRB3* genes increased by 16.64-fold and 54.53-fold respectively in the high group, while a down-regulation in the *BTG*2 transcript (0.15-fold) was observed in the low group (Figure not shown).

In the liver, high *ADRB3* and *BTG2* mRNA expression levels were detected in both the low or high groups. Significant up-regulation of the *ADRB*3 gene ($P<0.032$), corresponding to 60.59-fold in the low group, *BTG*2 gene ($P<0.024$), corresponding to 21.63-fold in the high group were noted (Figure not shown).

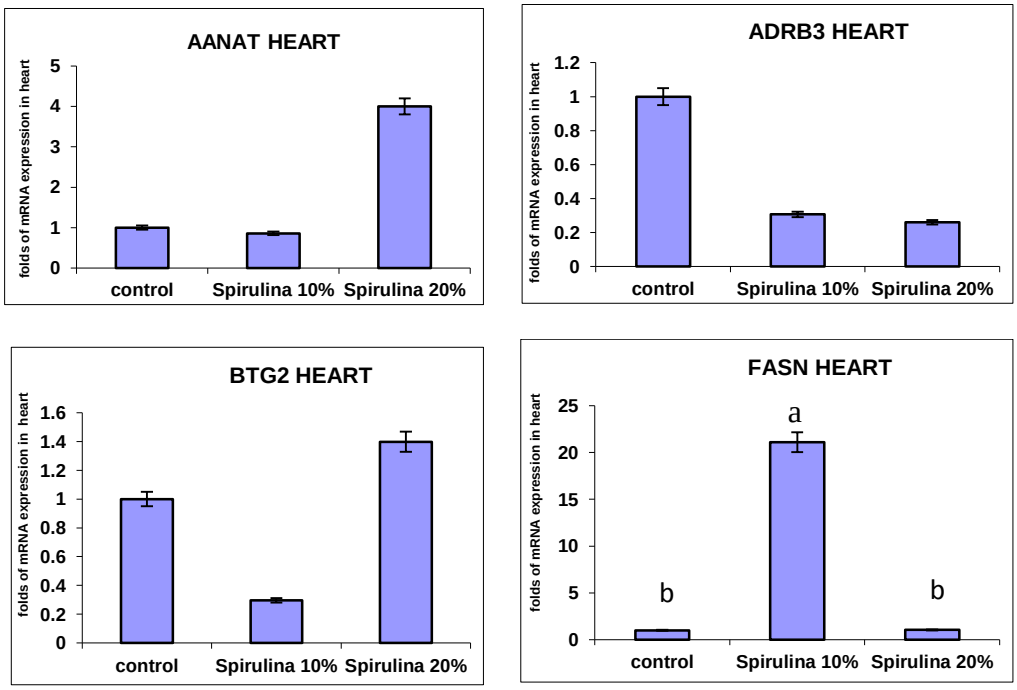

Figure 1. mRNA gene expressions in the heart of Australian prime lambs.

FASN is fundamental in enzyme regulation of the *de novo* synthesis step of lipogenesis and its main function is to catalyse the synthesis of palmitate from acetyl-CoA and malonyl-CoA, in the presence of NADPH, into long-chain FAs (Berndt *et al*., 2007; Byrne *et al*., 2005, Boizard *et al*., 1998). In the heart, the low level of *Spirulina* supplementation increased the transcription levels of the *FASN* gene leading to an increase in omega-6 (ω-6) and omega-9 (ω-9) PUFA and a decrease in saturated FA and a concomitant increase in PUFA in the heart of supplemented lambs.

In the kidney, significant expression of the *AANAT* encoded proteins accelerated the rate-limiting step in the synthesis of melatonin from serotonin (Coon *et al*., 1999, Reiter *et al*., 2014). Melatonin is a hormone that controls the function of the circadian clock, which regulates activity and sleep (Coon *et al*., 1999, Reiter *et al*., 2014). Spanish scientists discovered that melatonin consumption stimulated browning of white fat tissue in rats (Jiménez-Aranda *et al*., 2013). Brown fat burns, rather than stores, calories. Thus, melatonin has an anti-obesity effect and its metabolism protects against oxidative degradation of PUFA (Jiménez-Aranda *et al*., 2013, Reiter *et al*., 2014).In agreement, our fatty acid results (Kashani *et al*. 2015b) demonstrated that medium-level *Spirulina* supplementation significantly increased the ω-3 and ω-6 PUFA composition in all tissues and organs. The *ADRB3* gene plays a key role in regulating mammalian energy storage and expenditure (Malau-Aduli and Kashani 2015). It is also a principal mediator of the lipolytic and thermogenetic effects of high catecholamine (Forrest *et al*., 2007, Wu *et al*., 2011). The primary role of this receptor is in the regulation of resting metabolic rate and lipolysis (Forrest *et al*., 2003). Given that *ADRB3* encodes proteins regulating mammalian energy storage and expenditure by mediating effects from the sympathetic nervous system (Hu *et al*., 2010, Wu *et al*., 2012), our observations suggest an intricate genetics-nutrition interaction underpinning transcription at the molecular level that can be dietarily manipulated to achieve healthy FA composition outcomes.

BTG2 belongs to the anti-proliferative gene family and has been shown to be involved in cell growth, differentiation and survival (Mo *et al*., 2011), muscle fibre size, intramuscular fat deposition and weight loss (Kamaid and Giráldez, 2008, Sasaki *et al*., 2006). This seems to suggest that dietary *Spirulina* supplementation increases metabolic rate and lipolysis in the liver through up-regulation of *ADRB3* and simultaneously induces a decline in preadipocyte proliferation, an increase in energy expenditure, and a decline in energy uptake in adipocytes, ultimately enhancing ω-3 and ω-6 PUFA contents in the liver.

The results presented here demonstrated that mRNA expression levels of *AANAT*, *ADRB3*, *BTG2* and *FASN* in the heart, kidney, and liver are likely influenced by dietary *Spirulina* supplementation level. Our results show that genes involved in fatty acid metabolism in the kidney and liver are more sensitive to *Spirulina* supplementation than in the heart. These findings support the use of a low level of dietary *Spirulina* supplementation for optimal increase in healthy omega-3 and omega-6 fatty acid contents of organs among Australian crossbred sheep.

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