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EFFECT OF LEVEL OF *SPIRULINA* SUPPLEMENTATION ON THE FATTY ACID COMPOSITIONS OF ADIPOSE, MUSCLE, HEART, KIDNEY AND LIVER TISSUES IN AUSTRALIAN DUAL-PURPOSE LAMBS*

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Abstract

This study investigated the effect of level of *Spirulina* supplementation on the fatty acid (FA) compositions of subcutaneous adipose, *longissimus dorsi* muscle, kidney, heart, and liver tissues in purebred and crossbred Australian Merino sheep. Forty-eight lambs sired by Black Suffolk, White Suffolk, Dorset and Merino rams were assigned into 4 treatment groups of daily *Spirulina* supplementation levels per lamb of 0 mL (control), 50 mL (low), 100 mL (medium) and 200 mL (high) referred to as 0, 5, 10 and 20% groups. The lambs were slaughtered after 9 weeks of supplementation and heart, kidney, adipose, liver and muscle tissue samples were collected. The results demonstrated significant variations in growth and body conformation traits and tissue and organ FA composition in response to the *Spirulina* supplementation. The medium-level *Spirulina* treatment group increased the ω -3 and ω -6 polyunsaturated fatty acid (PUFA) composition in all tissues and organs significantly. The results suggest the use of medium level (100 mL/head/day) of *Spirulina* supplementation in order to increase lamb production with more ω -3 and ω -6 PUFA and therefore higher nutritional meat quality.

Key words: *Spirulina*, fatty acids, sheep, liver, kidney

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Spirulina platensis is a cyanobacterium (blue-green alga) commercially produced as a nutritional supplement for humans and as a feed ingredient for livestock (Holman and Malau-Aduli, 2013). *Spirulina* is produced mainly from two species of cyanobacteria: *Arthrospira platensis* and *Arthrospira maxima*. *Spirulina platensis* is regarded as a desirable supplement because it contains 60–70% protein and is a good source of essential vitamins and minerals (Holman and Malau-Aduli, 2013). In the dual-purpose prime lamb industry, accomplishing a higher quality of lamb product is central to economic success. When it comes to making purchasing decisions, carcass characteristics and meat quality are significant standards for the industry and consumers (Alfaia et al., 2009; Kouba and Mouro, 2011; Smet et al., 2004). In red meat, the fatty acid composition and cholesterol levels have received considerable attention due to their significance in human health and product quality (Mapiye et al., 2011; Woods and Fearon, 2009).

There are two types of factors that influence both type and composition of lipids in animal products: extrinsic and intrinsic factors such as age, genotype, gender, feeding and temperature. In recent years, there has been increased research interest in manipulating the fatty acid composition of meat. Nowadays consumers prefer healthy, naturally produced products, which has generated a lot of research interest to produce foods of higher nutritional quality, including meats (Mapiye et al., 2011; Moibi and Christopherson, 2001; Woods and Fearon, 2009). Modification of animal diets using bioactive feed supplements such as *Spirulina* is one strategy for producing such foods (Doreau et al., 2010; Iwata et al., 1990).

Although *Spirulina* is the subject of recent research investigations in sheep (Kashani et al., 2015; Holman et al., 2014 a, 2014 b, 2014 c; Holman and Malau-Aduli, 2014; Holman and Malau-Aduli, 2013; Holman et al., 2012), none of these papers has dealt specifically with fatty acid composition. Therefore, the major objective of this study was to investigate the effect of the level of *Spirulina* supplementation on the fatty acid (FA) composition of subcutaneous adipose, *longissimus dorsi* muscle, heart, kidney and liver tissues in dual-purpose Australian lambs. It was hypothesized that *Spirulina* would affect lamb tissues and organ FA composition.

Material and methods

This study was carried out at the University of Tasmania (UTAS) Farm, Cambridge, Tasmania, Australia. All experimental processes conformed to the UTAS Animal Ethics Committee guidelines, the 1993 Tasmania Animal Welfare Act, and the 2004 Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Animal management, slaughter, and sampling

At the UTAS Farm, Merino, Dorset, Black Suffolk, and White Suffolk terminal sires were mated over two consecutive years with purebred Merino ewes using a 1:100 ram-to-ewe mating ratio. All first progeny were identified using National

Livestock Identification ear tags at birth and were weaned onto ryegrass pastures at three months of age. At 6 months of age average weaning weight of 30 kg, 48 lambs were allocated into a feeding trial using ryegrass pasture. Each treatment group had twelve lambs balanced by sire breed (Black Suffolk, White Suffolk, Dorset, and Merinos), sex (ewe, wether), and *Spirulina* supplementation level (Control – unsupplemented, Low, Medium and High).

Spirulina powder was purchased (TAAU, Darwin, Northern Territory, Australia) and made into a water suspension using a 1:10 weight/volume ratio of *Spirulina* (g) to water (ml). The control treatment was drenched with water only without any *Spirulina* (0% wt:vol), Low level of supplementation was 50 mL/head/day of 1 g *Spirulina* powder dissolved in 10 mL of water (5% wt:vol), Medium (100 mL/head/day of 1 g *Spirulina* powder dissolved in 10 mL of water (10% wt:vol) and High (200 mL/head/day of 2 g of *Spirulina* dissolved in 10 mL of water (20% wt:vol). Each lamb was directly provided with its assigned *Spirulina* supplementation level daily via oral drenching. At weekly intervals, each lamb was individually assessed for chest girth (CG), withers height (WH), body length (BL), body condition score (BCS) and liveweight (BWT) measurements. CG was the body circumference measured at just behind the forelegs (Afolayan et al., 2006). WH was the distance between the highest peak over the scapulae and the ground (Sowande and Sobola, 2008). BL refers to the span between the base of the neck, the vertebrae between the scapulae, to the far point of the pubic bone (Sowande and Sobola, 2008). BCS was subjectively measured (Phythian et al., 2012), always by the same researcher, gauging fat depth on a 0–5 point scale as described by McLeod et al. (2010). BWT was monitored using an electronic walk-over weighing scale equipped with an automatic sheep ID scanning digitally downloadable data capability. Body conformation measurements in centimetres were taken using the same measuring tape. During assessment it was ensured that lambs were gently restrained in a relaxed state on all four legs with their heads comfortably erect. All experimental lambs were slaughtered at the completion of the feeding trial (finishing weight ranging from 40 to 45 kg) at a commercial abattoir (Gretna Quality Meats, Gretna, Tasmania, Australia). Subcutaneous adipose fat, kidney, liver, heart, and *longissimus dorsi* muscle tissue samples were immediately removed from each carcass and frozen in liquid nitrogen, transported to the laboratory, and stored at -20°C for further analysis.

Lipid extraction and GC analysis

All tissue samples (subcutaneous adipose, kidney, heart, liver, and muscle) were extracted using a modified Bligh and Dyer protocol (Bligh and Dyer, 1959). This involved a single-phase overnight extraction using CHCl_3 :MeOH:H₂O (1:2:0.8 v/v), followed by phase separation with the addition of CHCl_3 :saline H₂O (1:1 v/v). The total lipid extract was obtained by rotary evaporation of the lower chloroform phase.

An aliquot of total lipid extracted from each sample was transmethylated in MeOH:CHCl₃:HCl (10:1:1 v/v) for 2 h at 80°C. Milli-Q H₂O (1 ml) was then added before FA methyl esters (FAME) were extracted with hexane:chloroform (4:1 v/v) and reduced under a nitrogen stream, and a known concentration of an internal injection standard (19:0 FAME) was added. An Agilent Technologies 7890B gas

chromatograph (GC) (Palo Alto, California, USA) equipped with an Equity™-1 fused silica capillary column (15 m × 0.1 mm internal diameter and 0.1-μm film thickness), a flame ionisation detector, a split/splitless injector, and an Agilent Technologies 7683 B Series autosampler was used in the analysis.

Samples were injected in splitless mode and carried by helium gas at an oven temperature of 120°C. Post injection, the oven temperature was increased to 270°C at 10°C/min, and then to 310°C at 5°C/min. Peaks were quantified by Agilent Technologies ChemStation software (Palo Alto, California, USA). FA identities were confirmed using GC-mass spectrometric (GC/MS) analysis. These analyses were performed using a Finnigan Thermoquest GCQ GC-MS fitted with an on-column injector with Thermoquest Xcalibur software (Austin, Texas, USA). The GC had an HP-5 cross-linked methyl silicone-fused silica capillary column (50 m × 0.32 mm internal diameter). The carrier gas used was helium, with operating conditions previously described (Miller et al., 2006).

Statistical analysis

Individual FAs and the summations of saturated, monounsaturated, and polyunsaturated FAs were computed as percentages of total FAs. Statistical Analysis System software (SAS, 2009) was used to analyse the data, and summary statistics were computed with means, standard deviations, and minimum and maximum values to check for errors and outliers. Repeated measures analysis of variance in general linear models procedure (PROC GLM), using Statistical Analysis System (SAS, 2009) was then carried out by fitting the level of *Spirulina* supplementation, sire breed, sex and tissue as fixed effects in the model and using FA values as dependent variables (14:0, 15:0, 16:1ω9c, 16:1ω7c, 16:0, 17:0, 18:2ω6, 18:3ω3, 18:1ω9, 18:1ω7c, 18:1ω7t, 18:0, 20:4ω6, 20:5ω3, 20:3ω6, 20:4ω3, 20:2ω6, 20:0, 22:5ω6, 22:6ω3, 22:5ω3, 22:0, 23:0, 24:0, ΣSFA, ΣMUFA, ΣPUFA, Σω-3 PUFA, Σω-6 PUFA). Bonferroni's probability pairwise comparison test was used to separate mean differences, with the level of significance defined as P<0.05.

Results

Spirulina supplementation and phenotypic data

Spirulina supplementation enabled sheep to grow longer bodies (BL) than the control group (P<0.015). Furthermore, lambs in the high (20%) *Spirulina* supplementation treatment group had a greater body condition score (BCS) than the medium (10%), low (5%), and control (0%) treatments (P<0.001). It was observed that the sheep receiving medium *Spirulina* supplementation had the greatest body weight (BWT) of 41.9 kg (P<0.018), but no differences between the high, low, and control treatment groups were observed. The phenotypic results are shown in Table 2.

Spirulina and tissue fatty acids

The fatty acid composition of *Spirulina* analysed by GC-MS is listed in Table 1. The *Spirulina* diet had a significant effect on subcutaneous adipose, *longissimus*

dorsi muscle, heart, kidney, and liver tissue FA compositions of 14:0, 15:0, 16:1 ω 7c, 16:0, 17:0, 18:2 ω 6, 18:1 ω 9, 18:1 ω 7c, 20:5 ω 3, 20:3 ω 6, 20:4 ω 3, 20:2 ω 6, 20:0, 22:5 ω 6, 22:6 ω 3, Σ SFA, and $\Sigma\omega$ -6. In addition, the *Spirulina* diet significantly affected the profile of 16:0, 18:1 ω 9, 20:5 ω 3, and 22:5 ω 6 in two or more tissues (Table 3).

Table 1. The fatty acid composition of *Spirulina*

Fatty acid	Mean (% total FA)	SEM
16:0 Palmitic acid	24.8	1.4
16:1 ω 9c Palmitoleic acid	3.7	0.5
17:0 Heptadecanoic acid, or margaric acid	1.7	0.1
18:0	6.3	0.9
18:1 ω 9 Oleic acid	9.8	1.1
18:2 ω 6 Linoleic acid	12.2	1.4
18:3 ω 3 α -Linolenic acid	4.46	0.3
18:3 ω 6 γ -Linolenic acid	17.2	2.3
20:0 Arachidic acid	2.1	0.2
20:2 ω 6 Eicosadienoic acid	1.9	0.4
20:3 ω 6 Dihomo- γ -linolenic acid (DGLA)	2.2	0.2
20:5 ω 3 Eicosapentaenoic acid	1.95	0.1

Table 2. Least square means (LSM) of chest girth, wither height, body length, body condition score, live weight and average daily gain in *Spirulina*-supplemented lambs

	<i>Spirulina</i> treatments								P-value
	control (0)		low (50 mL/head/day)		medium (100 mL/head/day)		high (200 mL/head/day)		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
CG (cm)	95.0	0.6	95.2	0.4	95.6	0.6	96.1	0.7	0.376
WH (cm)	62.9	0.4	62.5	0.6	62.7	0.4	63.1	0.3	0.669
BL (cm)	65.7 b	0.4	65.6 b	0.4	66.6 a	0.4	66.8 a	0.4	0.015*
BCS (0–5)	3.2 b	0.1	3.1 b	0.1	3.3 b	0.0	3.4 a	0.1	0.001***
BWT (kg)	40.6 b	0.7	40.7 b	0.4	41.9 a	0.7	40.8 b	0.6	0.018*
ADG (kg/d)	0.1	0.0	0.1	0.0	0.2	0.0	0.1	0.0	0.759

Column means within a fixed effect bearing different letters significantly differ ($P < 0.05$). Chest girth (CG), withers height (WH), body length (BL), body condition score (BCS), body weight (BWT), and average daily weight gain (ADG). Level of significance: * significant ($P < 0.05$), ** highly significant ($P < 0.01$), and *** very highly significant ($P < 0.001$).

Tissue fatty acid content

Adipose: In the subcutaneous adipose tissue, the level of 16:1 ω 7c composition was found to be higher by 1.2% in the tissues of animals that received medium and high levels of *Spirulina* compared to the control (0.9%) and low (1.1%) levels of supplementation (Table 4). The highest composition of 18:2 ω 6 (linoleic acid) was discovered in the tissues associated with the medium level of *Spirulina* supplementation (1.8%) with a significant difference ($P < 0.05$), in contrast to the compositions for the other supplementation levels, which did not differ significantly (Table 4).

Table 3. Level of significance (P-values) and tissue variation in subcutaneous adipose, heart, kidney, liver and muscle fatty acid composition

Fatty acid profile	Adipose	Muscle	Heart	Kidney	Liver
14:0 Myristic acid	0.760	0.252	0.177	0.900	0.001
15:0 Pentadecanoic acid	0.661	0.969	0.045	0.453	0.471
16:1 ω 9c Palmitoleic acid	0.640	0.575	0.313	0.616	0.592
16:1 ω 7c	0.043	0.651	0.271	0.510	0.241
16:0 Palmitic acid	0.021	0.014	0.047	0.441	0.326
17:0 Heptadecanoic or margaric acid	0.369	0.004	0.060	0.241	0.106
18:2 ω 6 Linoleic acid	0.027	0.665	0.899	0.233	0.972
18:3 ω 3 α -Linolenic acid	0.879	0.474	0.185	0.241	0.587
18:1 ω 9 Oleic acid	0.441	0.619	0.047	0.041	0.289
18:1 ω 7c Vaccenic acid	0.424	0.029	0.536	0.612	0.863
18:1 ω 7t	0.140	0.702	0.644	0.628	0.139
18:0	0.870	0.649	0.575	0.141	0.904
20:4 ω 6	0.398	0.860	0.582	0.792	0.571
20:5 ω 3 Eicosapentaenoic acid	0.048	0.004	0.614	0.475	0.048
20:3 ω 6 Dihomo- γ -linolenic acid (DGLA)	0.722	0.722	0.223	0.192	0.036
20:4 ω 3 Eicosatetraenoic acid	.	.	0.040	0.705	.
20:2 ω 6 Eicosadienoic acid	.	.	0.006	0.892	.
20:0 Arachidic acid	0.003	0.597	0.357	0.953	0.757
22:5 ω 6 Docosapentaenoic acid	0.341	0.531	0.307	0.011	0.010
22:6 ω 3 Docosahexaenoic acid (DHA)	0.951	0.914	0.552	0.004	0.430
22:5 ω 3 DPA-3	.	.	0.935	0.267	0.090
22:0	.	.	0.259	0.556	0.811
23:0	.	0.419	.	0.919	0.791
24:0	.	0.795	0.584	0.605	0.844
Σ SFA	0.414	0.396	0.713	0.035	0.487
Σ MUFA	0.296	0.419	0.372	0.310	0.405
Σ PUFA	0.373	0.812	0.441	0.677	0.237
$\Sigma\omega$ -3	0.933	0.462	0.331	0.321	0.107
$\Sigma\omega$ -6	0.040	0.811	0.774	0.512	0.784

* Σ SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; Σ MUFA is the sum of 14:1 ω 5, 15:1 ω 6, 16:1 ω 9, 16:1 ω 7, Br17:1, 17:1 ω 8+a17:0, 17:1, 18:1 ω 9, 18:1 ω 7, 18:1 ω 5, 18:1, 19:1, 20:1 ω 11, 20:1 ω 9, 20:1 ω 7, 20:1 ω 5, 22:1 ω 9, 22:1 ω 11, 22:1 ω 7, 24:1 ω 11, 24:1 ω 9, 24:1 ω 7; Σ PUFA is the sum of 16:3+16:4, 16:2, 18:4 ω 3, 18:3 ω 6, 18:2 ω 6, 18:3 ω 3, 20:4 ω 3, 20:4 ω 6, 20:5 ω 3, 20:3 ω 6, 20:2 ω 6, 21:5 ω 3, 22:6 ω 3, 22:5 ω 3, 22:5 ω 6, 22:4 ω 6, 24:6 ω 3, 24:5 ω 3; $\Sigma\omega$ -3 PUFA is the sum of 18:3 ω 3, 18:4 ω 3, 20:4 ω 3, 20:5 ω 3, 21:5 ω 3, 22:6 ω 3, 22:5 ω 3, 24:6 ω 3, 24:5 ω 3; $\Sigma\omega$ -6 PUFA is the sum of 18:2 ω 6, 18:3 ω 6, 20:4 ω 6, 20:3 ω 6, 20:2 ω 6, 22:5 ω 6, 22:4 ω 6. Level of significance: * significant (P<0.05), ** highly significant (P<0.01), and *** very highly significant (P<0.001).

For 20:5 ω 3 (eicosapentaenoic acid), the highest composition of 0.2 (Table 4) was observed for the medium supplementation levels, which significantly differed (P<0.05) from the low and control supplementation levels. However, no significant difference was found compared to the high supplementation level (Table 4). In the adipose tissue, the highest level of 20:0 composition (arachidic acid) was 0.2% (Table 4), which was found in tissues associated with low level of supplementation with a significant difference (P<0.01). This was followed by 0.1% for the control and

high supplementation levels (Table 4). The lowest level of arachidic acid in adipose tissues was recorded for the medium level of supplementation. The medium level of supplementation produced the highest $\sum\omega$ -6 composition of 2.1 (Table 4), which significantly differed from other tissues. The $\sum\omega$ -6 composition was observed to be significantly higher for both low and high supplementations compared to the control group (Table 4).

Table 4. Subcutaneous adipose tissue fatty acid composition (% total fatty acids), standard error of mean (SEM) and number of lambs (n)^{a, b, c}

Fatty acids	<i>Spirulina</i> supplementation treatment group							
	control (n=12)		low (n=12)		medium (n=12)		high (n=12)	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM
14:0	3.0	0.2	3.1	0.1	2.2	0.4	2.6	0.4
15:0	0.7	0.0	0.6	0.1	0.6	0.1	0.6	0.1
16:1 ω 9c	0.4	0.0	0.3	0.0	0.3	0.0	0.4	0.0
16:1 ω 7c	0.9 b	0.2	1.1 b	0.0	1.2 a	0.0	1.2 a	0.0
16:0	24.1	0.1	25.4	0.0	22.7	0.1	24.3	0.1
17:0	1.9	0.1	2.1	0.1	1.8	0.1	2.0	0.1
18:2 ω 6	1.6 b	0.0	1.6 b	0.0	1.8 a	0.1	1.5 b	0.1
18:3 ω 3	1.5	0.1	1.4	0.2	1.4	0.1	1.4	0.1
18:1 ω 9	32.4	1.6	29.4	2.4	34.7	2.0	32.7	1.3
18:1 ω 7c	1.3	0.1	1.1	0.0	1.3	0.1	1.3	0.0
18:1 ω 7t	4.0	0.3	2.7	0.2	3.6	0.3	3.6	0.2
18:0	23.3	1.4	26.9	1.2	24.4	3.3	23.1	1.4
20:4 ω 6	0.01	0.01	0.1	0.01	0.01	0.01	0.01	0.01
20:5 ω 3	0.01	0.01	0.01	0.01	0.2 a	0.0	0.1 b	0.1
20:2 ω 6	0.1	0.01	0.01	0.01	0.01	0.01	0.01	0.01
20:0	0.1 b	0.0	0.2 a	0.01	0.01 c	0.01	0.1 b	0.01
22:5 ω 6	0.1	0.0	0.2	0.1	0.0	0.0	0.1	0.1
22:6 ω 3	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1
\sum SFA	53.0	0.2	53.8	0.1	56.4	0.5	53.7	0.8
\sum MUFA	43.7	0.6	42.6	0.4	37.1	0.4	42.6	0.4
\sum PUFA	3.3	0.1	3.6	0.1	6.5	0.2	3.7	0.1
$\sum\omega$ -3	1.6	0.1	1.5	0.1	1.4	0.1	1.6	0.2
$\sum\omega$ -6	1.6 c	0.01	1.7 b	0.2	2.1 a	0.1	1.8 b	0.1

^a Means with different letters – a, b, c within rows significantly differ ($P < 0.01$).

^b \sum SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; \sum MUFA is the sum of 14:1 ω 5, 15:1 ω 6, 16:1 ω 9, 16:1 ω 7, Br17:1, 17:1 ω 8+a17:0, 17:1, 18:1 ω 9, 18:1 ω 7, 18:1 ω 5, 18:1, 19:1, 20:1 ω 11, 20:1 ω 9, 20:1 ω 7, 20:1 ω 5, 22:1 ω 9, 22:1 ω 11, 22:1 ω 7, 24:1 ω 11, 24:1 ω 9, 24:1 ω 7; \sum PUFA is the sum of 16:3+16:4, 16:2, 18:4 ω 3, 18:3 ω 6, 18:2 ω 6, 18:3 ω 3, 20:4 ω 3, 20:4 ω 6, 20:5 ω 3, 20:3 ω 6, 20:2 ω 6, 21:5 ω 3, 22:6 ω 3, 22:5 ω 3, 22:5 ω 6, 22:4 ω 6, 24:6 ω 3, 24:5 ω 3; $\sum\omega$ -3 PUFA is the sum of 18:3 ω 3, 18:4 ω 3, 20:4 ω 3, 20:5 ω 3, 21:5 ω 3, 22:6 ω 3, 22:5 ω 3, 24:6 ω 3, 24:5 ω 3; $\sum\omega$ -6 PUFA is the sum of 18:2 ω 6, 18:3 ω 6, 20:4 ω 6, 20:3 ω 6, 20:2 ω 6, 22:5 ω 6, 22:4 ω 6.

^c FA not found (%total FA = 0) were 20:3 ω 6, 20:4 ω 3, 22:5 ω 3, 22:0, 23:0, 24:0.

Longissimus dorsi muscle: The level of palmitic acid (16:0) in the muscle tissue of control group was higher than that for the low, medium, and high levels

of supplementation with a significant difference ($P < 0.05$) (Table 5). For 17:0 (heptadecanoic acid) muscle composition, the low level of supplementation differed significantly ($P < 0.01$) from other supplementation levels, having the highest composition of 1.6% (Table 5). Both the low and high levels of supplementation had the highest 18:1 ω 7c muscle composition (1.5%), which significantly differed ($P < 0.05$) from the control and medium levels (Table 5). For 20:5 ω 3 (eicosapentaenoic acid), the highest muscle composition of 0.5% was found with the medium and high supplementation levels, which significantly differed ($P < 0.05$) from the control and low-level supplementation groups (Table 5).

Table 5. *Longissimus dorsi* muscle fatty acid composition (% total fatty acids), standard error of mean (SEM) and number of lambs (n)^{a, b, c}

Spirulina supplementation treatments								
Fatty acids	control (n=12)		low (n=12)		medium (n=12)		high (n=12)	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM
14:0	1.8	0.2	1.1	0.7	1.8	0.2	1.9	0.2
15:0	0.5	0.0	0.4	0.1	0.5	0.1	0.4	0.0
16:1 ω 9c	0.3	0.0	0.3	0.0	0.3	0.0	0.2	0.0
16:1 ω 7c	1.1	0.1	1.1	0.3	1.1	0.1	1.3	0.1
16:0	23.8 a	0.1	22.2 b	0.1	22.0 b	0.0	22.6 b	0.2
17:0	1.4	0.0 b	1.6 a	0.1	1.3 b	0.0	1.3 b	0.1
18:2 ω 6	4.5	0.6	3.8	0.6	3.7	0.3	4.1	0.4
18:3 ω 3	2.0	0.1	1.7	0.2	2.0	0.1	2.0	0.1
18:1 ω 9	35.5	1.0	36.4	1.2	36.5	0.7	35.5	1.5
18:1 ω 7c	1.4 b	0.1	1.5 a	0.1	1.5 ab	0.0	1.5 a	0.1
18:1 ω 7t	3.1	0.2	2.7	0.1	2.8	0.1	2.8	0.2
18:0	20.1	0.7	20.6	1.2	19.2	0.8	20.0	0.7
20:4 ω 6	0.7	0.2	0.5	0.0	0.7	0.1	0.7	0.1
20:5 ω 3	0.1 b	0.1	0.2 b	0.1	0.5 a	0.1	0.5 a	0.1
20:3 ω 6	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0
20:0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0
22:5 ω 6	0.1	0.0	0.1	0.0	0.1	0.1	0.0	0.0
22:6 ω 3	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.0
22:5 ω 3	0.2	0.1	0.2	0.0	0.3	0.1	0.4	0.1
Σ SFA	46.6	1.2	48.0	1.0	45.7	1.0	47.1	1.3
Σ MUFA	44.8	1.0	45.1	1.1	46.4	1.0	44.7	1.3
Σ PUFA	8.6	1.1	6.9	0.7	7.9	0.6	8.3	0.8
$\Sigma\omega$ -3	2.9	0.3	2.1	0.2	3.0	0.2	3.0	0.3
$\Sigma\omega$ -6	5.5	0.8	4.5	0.6	4.7	0.4	5.0	0.6

^a Means with different letters – a, b within rows significantly differ ($P < 0.01$).

^b Σ SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; Σ MUFA is the sum of 14:1 ω 5, 15:1 ω 6, 16:1 ω 9, 16:1 ω 7, Br17:1, 17:1 ω 8+a17:0, 17:1, 18:1 ω 9, 18:1 ω 7, 18:1 ω 5, 18:1, 19:1, 20:1 ω 11, 20:1 ω 9, 20:1 ω 7, 20:1 ω 5, 22:1 ω 9, 22:1 ω 11, 22:1 ω 7, 24:1 ω 11, 24:1 ω 9, 24:1 ω 7; Σ PUFA is the sum of 16:3+16:4, 16:2, 18:4 ω 3, 18:3 ω 6, 18:2 ω 6, 18:3 ω 3, 20:4 ω 3, 20:4 ω 6, 20:5 ω 3, 20:3 ω 6, 20:2 ω 6, 21:5 ω 3, 22:6 ω 3, 22:5 ω 3, 22:5 ω 6, 22:4 ω 6, 24:6 ω 3, 24:5 ω 3; $\Sigma\omega$ -3 PUFA is the sum of 18:3 ω 3, 18:4 ω 3, 20:4 ω 3, 20:5 ω 3, 21:5 ω 3, 22:6 ω 3, 22:5 ω 3, 24:6 ω 3, 24:5 ω 3; $\Sigma\omega$ -6 PUFA is the sum of 18:2 ω 6, 18:3 ω 6, 20:4 ω 6, 20:3 ω 6, 20:2 ω 6, 22:5 ω 6, 22:4 ω 6.

^c FA not found (%total FA = 0) were 20:4 ω 3, 20:2 ω 6, 22:0, 23:0, 24:0.

Table 6. Heart fatty acid composition (% total fatty acids), standard error of mean (SEM) and number of lambs (n)^{a, b}

Fatty acids	<i>Spirulina</i> treatments							
	control (n=12)		low (n=12)		medium (n=12)		high (n=12)	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM
14:0	0.7 b	0.2	1.2 a	0.4	0.8 ab	0.2	0.5 b	0.1
15:0	0.4 a	0.01	0.2	0.01 b	0.01	0.01 b	0.4	0.01a
16:1 ω 9c	0.2	0.01	0.2	0.01	0.3	0.01	0.2	0.01
16:1 ω 7c	0.4	0.1	0.1	0.1	0.4	0.1	0.3	0.1
16:0	15.1 a	0.1	14.7 ab	0.6	14.1 b	0.1	15.0 a	0.4
17:0	1.3	0.0	1.5	0.1	1.3	0.0	1.4	0.0
18:2 ω 6	17.1	1.2	15.5	1.6	16.5	1.5	15.7	1.4
18:3 ω 3	3.6	0.4	2.1	0.1	3.4	0.3	3.0	0.2
18:1 ω 9	18.1 b	0.4	19.9 a	0.2	19.8 a	0.1	19.0 ab	0.1
18:1 ω 7c	1.9	0.1	2.0	0.2	2.0	0.1	1.9	0.1
18:1 ω 7t	2.1	0.2	1.7	0.2	2.3	0.2	2.2	0.2
18:0	19.3	1.0	21.0	1.8	21.5	1.4	21.6	1.3
20:4 ω 6	5.6	0.5	4.6	0.8	4.1	0.6	4.8	0.7
20:5 ω 3	3.1	0.6	1.5	0.2	2.3	0.2	2.6	0.6
20:3 ω 6	0.6	0.01	0.5	0.1	0.5	0.1	0.6	0.01
20:4 ω 3	0.1 b	0.01	0.2 a	0.01	0.1 b	0.01	0.1 b	0.01
20:2 ω 6	0.01 b	0.01	0.01 b	0.01	0.1 a	0.01	0.01 b	0.01
20:0	0.1 c	0.01	0.3 a	0.01	0.1 c	0.01	0.2 b	0.01
22:5 ω 6	0.01	0.01	0.1	0.01	0.01	0.01	0.01	0.01
22:6 ω 3	1.3	0.2	0.9	0.2	0.7	0.1	1.0	0.2
22:5 ω 3	1.9	0.3	1.6	0.3	1.2	0.2	1.5	0.2
22:0	0.3	0.1	0.2	0.01	0.1	0.01	0.3	0.1
23:0	0.2	0.1	0.3	0.01	0.1	0.01	0.3	0.01
24:0	0.2	0.01	0.2	0.01	0.1	0.01	0.2	0.01
Σ SFA	37.8	1.2	41.1	3.3	40.1	1.8	40.7	1.5
Σ MUFA	28.2	0.9	30.9	1.4	30.3	0.9	29.2	1.1
Σ PUFA	34.0	1.4	27.9	3.3	29.6	2.5	30.1	2.0
$\Sigma\omega$ -3	10.0	1.0	6.3	0.8	7.8	0.6	8.3	0.9
$\Sigma\omega$ -6	23.4	1.3	21.0	2.5	21.4	2.0	21.4	1.7

^a Means with different letters – a, b, c within rows significantly differ (P<0.01).

^b Σ SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; Σ MUFA is the sum of 14:1 ω 5, 15:1 ω 6, 16:1 ω 9, 16:1 ω 7, Br17:1, 17:1 ω 8+a17:0, 17:1, 18:1 ω 9, 18:1 ω 7, 18:1 ω 5, 18:1, 19:1, 20:1 ω 11, 20:1 ω 9, 20:1 ω 7, 20:1 ω 5, 22:1 ω 9, 22:1 ω 11, 22:1 ω 7, 24:1 ω 11, 24:1 ω 9, 24:1 ω 7; Σ PUFA is the sum of 16:3+16:4, 16:2, 18:4 ω 3, 18:3 ω 6, 18:2 ω 6, 18:3 ω 3, 20:4 ω 3, 20:4 ω 6, 20:5 ω 3, 20:3 ω 6, 20:2 ω 6, 21:5 ω 3, 22:6 ω 3, 22:5 ω 6, 22:4 ω 6, 24:6 ω 3, 24:5 ω 3; $\Sigma\omega$ -3 PUFA is the sum of 18:3 ω 3, 18:4 ω 3, 20:4 ω 3, 20:5 ω 3, 21:5 ω 3, 22:6 ω 3, 22:5 ω 3, 24:6 ω 3, 24:5 ω 3; $\Sigma\omega$ -6 PUFA is the sum of 18:2 ω 6, 18:3 ω 6, 20:4 ω 6, 20:3 ω 6, 20:2 ω 6, 22:5 ω 6, 22:4 ω 6.

Heart: The lowest 15:0 (pentadecanoic acid) fatty acid composition in heart tissue of 0.2% was discovered in tissues supplemented with low levels, while the compositions for other supplementations did not differ significantly (Table 6). The 16:0 (palmitic acid) fatty acid composition differed (P<0.05) in heart tissues supplemented with low and medium levels compared to the control (Table 6). The medium level of supplementation was associated with the lowest 16:0 composition of 14.1%, followed by the low level (14.7%) (Table 6). Tissues supplemented with medium levels had the highest 20:2 ω 6 (eicosadienoic acid) composition of 0.1%,

which differed significantly ($P < 0.01$), whereas other supplementation compositions did not differ significantly (Table 6). The 18:1 ω 9 (oleic acid) heart composition was discovered to be higher for both the low and medium supplementation levels (Table 6). The 20:4 ω 3 (eicosatetraenoic acid) composition in heart tissue was found to be highest at 0.1% and significantly differed from the control, low, and medium levels of supplementations (Table 6).

Table 7. Kidney fatty acid composition (% total fatty acids), standard error of mean (SEM) and number of lambs (n)^{a, b}

Fatty acids	<i>Spirulina</i> treatments							
	control (n=12)		low (n=12)		medium (n=12)		high (n=12)	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM
14:0	0.2	0.1	0.2	0.2	0.1	0.1	0.2	0.1
15:0	0.3	0.0	0.2	0.1	0.3	0.1	0.3	0.0
16:1 ω 9c	0.2	0.0	0.1	0.1	0.2	0.0	0.2	0.1
16:1 ω 7c	0.3	0.0	0.3	0.1	0.4	0.1	0.3	0.1
16:0	19.2	0.8	19.0	1.3	19.3	1.7	18.2	0.3
17:0	1.4	0.1	1.6	0.1	1.3	0.1	1.3	0.1
18:2 ω 6	8.9	0.4	10.7	0.6	9.0	0.6	9.7	0.4
18:3 ω 3	1.9	0.3	1.6	0.4	4.4	2.3	2.1	0.3
18:1 ω 9	17.1 a	0.3	15.8 b	0.4	15.2 b	0.2	15.2 b	1.0
18:1 ω 7c	1.4	0.1	1.8	0.1	1.5	0.2	1.4	0.1
18:1 ω 7t	1.6	0.2	0.9	0.1	1.3	0.3	1.2	0.2
18:0	22.5	1.0	21.1	1.1	19.0	1.6	19.8	1.2
20:4 ω 6	7.9	1.0	9.4	1.5	8.4	1.3	10.3	1.3
20:5 ω 3	5.0	0.9	2.7	0.7	5.1	0.9	5.5	0.7
20:3 ω 6	0.6	0.1	0.7	0.1	0.5	0.1	0.7	0.1
20:4 ω 3	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
20:2 ω 6	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0
20:0	0.2	0.0	0.1	0.0	0.2	0.0	0.2	0.0
22:5 ω 6	0.1 b	0.1	0.1 b	0.01	0.9 a	0.07	0.5 a	0.1
22:6 ω 3	2.5 b	0.2	2.7 b	0.1	2.8 a	0.2	3.4 a	0.1
22:5 ω 3	2.7	0.3	2.6	0.3	2.6	0.4	3.2	0.2
22:0	1.0	0.1	0.9	0.2	1.5	0.3	1.0	0.1
23:0	0.2	0.1	0.2	0.1	0.3	0.1	0.2	0.1
24:0	0.8	0.1	0.9	0.3	1.3	0.4	0.7	0.1
Σ SFA	46.6	1.6	44.7	2.3	44.4	0.9	42.7	1.3
Σ MUFA	22.7	0.7	23.5	1.8	22.6	1.6	21.9	1.0
Σ PUFA	30.8 b	0.5	31.8 b	0.1	33.9 a	0.5	35.4 a	0.6
$\Sigma\omega$ -3	12.5	1.5	9.8	0.7	14.7	1.8	14.0	0.9
$\Sigma\omega$ -6	18.0	1.2	21.8	1.2	18.1	1.6	21.0	1.5

^aMeans with different letters – a, b within rows significantly differ ($P < 0.05$).

^b Σ SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; Σ MUFA is the sum of 14:1 ω 5, 15:1 ω 6, 16:1 ω 9, 16:1 ω 7, Br17:1, 17:1 ω 8+a17:0, 17:1, 18:1 ω 9, 18:1 ω 7, 18:1 ω 5, 18:1, 19:1, 20:1 ω 11, 20:1 ω 9, 20:1 ω 7, 20:1 ω 5, 22:1 ω 9, 22:1 ω 11, 22:1 ω 7, 24:1 ω 11, 24:1 ω 9, 24:1 ω 7; Σ PUFA is the sum of 16:3+16:4, 16:2, 18:4 ω 3, 18:3 ω 6, 18:2 ω 6, 18:3 ω 3, 20:4 ω 3, 20:4 ω 6, 20:5 ω 3, 20:3 ω 6, 20:2 ω 6, 21:5 ω 3, 22:6 ω 3, 22:5 ω 3, 22:5 ω 6, 22:4 ω 6, 24:6 ω 3, 24:5 ω 3; $\Sigma\omega$ -3 PUFA is the sum of 18:3 ω 3, 18:4 ω 3, 20:4 ω 3, 20:5 ω 3, 21:5 ω 3, 22:6 ω 3, 22:5 ω 3, 24:6 ω 3, 24:5 ω 3; $\Sigma\omega$ -6 PUFA is the sum of 18:2 ω 6, 18:3 ω 6, 20:4 ω 6, 20:3 ω 6, 20:2 ω 6, 22:5 ω 6, 22:4 ω 6.

Table 8. Liver fatty acid composition (% total fatty acids), standard error of mean (SEM), number of lambs^{a, b, c}

Fatty acids	Spirulina treatments							
	control (n=12)		low (n=12)		medium (n=12)		high (n=12)	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM
14:0	0.6	0.2 A	0.5	0.2 A	0.3	0.1 B	0.3	0.1 B
15:0	0.5	0.1	0.5	0.1	0.5	0.0	0.4	0.0
16:1 ω 9c	0.4	0.1	0.4	0.1	0.4	0.0	0.4	0.0
16:1 ω 7c	0.6	0.1	0.4	0.1	0.7	0.1	0.5	0.1
16:0	19.8	1.4	22.0	1.0	18.9	0.9	19.7	1.2
17:0	1.5	0.1	2.0	0.1	1.3	0.1	1.5	0.1
18:2 ω 6	6.4	0.5	6.2	0.7	6.4	0.4	6.4	0.5
18:3 ω 3	3.0	0.2	2.4	0.7	3.4	0.3	3.0	0.3
18:1 ω 9	21.0	1.1	25.6	1.1	22.0	1.0 b	22.1	1.0 b
18:1 ω 7c	1.3	0.1	1.3	0.1	1.3	0.0	1.2	0.0
18:1 ω 7t	2.4	0.4	2.0	0.1	1.8	0.3	2.4	0.3
18:0	22.5	2.6	23.9	1.8	21.9	1.4	23.3	1.3
20:4 ω 6	4.4	0.9	2.9	0.8	4.4	0.6	3.8	0.7
20:5 ω 3	1.0 b	0.6	3.1 a	0.4	3.5 a	0.5	2.8 a	0.6
20:3 ω 6	0.3 b	0.1	0.5 a	0.1	0.6 a	0.1	0.5 a	0.1
20:0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0
22:5 ω 6	0.1 b	0.01	0.1 b	0.09	1.1 a	0.01	0.1 b	0.01
22:6 ω 3	3.9	0.7	1.9	0.3	3.7	0.3	3.0	0.5
22:5 ω 3	3.3	0.5	1.6	0.4	3.8	0.4	3.3	0.5
22:0	0.2	0.1	0.1	0.0	0.2	0.1	0.2	0.1
23:0	0.1	0.1	0.2	0.1	0.2	0.1	0.2	0.1
24:0	0.2	0.1	0.1	0.0	0.2	0.1	0.2	0.1
Σ SFA	46.1	2.2	50.0	2.5	44.4	1.2	46.7	1.7
Σ MUFA	28.6	1.3	32.4	1.3	29.0	1.1	29.8	1.1
Σ PUFA	25.3	2.7	17.6	2.4	26.6	1.6	23.5	2.3
$\Sigma\omega$ -3	13.4 b	1.7	6.9 d	1.5	14.6 a	1.1	12.3 c	1.5
$\Sigma\omega$ -6	11.6	1.3	10.5	1.0	11.7	1.0	11.0	1.2

^a Means with different letters – a, b, c, d within rows significantly differ (P<0.05).

^b Σ SFA is the sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; Σ MUFA is the sum of 14:1 ω 5, 15:1 ω 6, 16:1 ω 9, 16:1 ω 7, Br17:1, 17:1 ω 8+a17:0, 17:1, 18:1 ω 9, 18:1 ω 7, 18:1 ω 5, 18:1, 19:1, 20:1 ω 11, 20:1 ω 9, 20:1 ω 7, 20:1 ω 5, 22:1 ω 9, 22:1 ω 11, 22:1 ω 7, 24:1 ω 11, 24:1 ω 9, 24:1 ω 7; Σ PUFA is the sum of 16:3+16:4, 16:2, 18:4 ω 3, 18:3 ω 6, 18:2 ω 6, 18:3 ω 3, 20:4 ω 3, 20:4 ω 6, 20:5 ω 3, 20:3 ω 6, 20:2 ω 6, 21:5 ω 3, 22:6 ω 3, 22:5 ω 3, 22:5 ω 6, 22:4 ω 6, 24:6 ω 3, 24:5 ω 3; $\Sigma\omega$ -3 PUFA is the sum of 18:3 ω 3, 18:4 ω 3, 20:4 ω 3, 20:5 ω 3, 21:5 ω 3, 22:6 ω 3, 22:5 ω 3, 24:6 ω 3, 24:5 ω 3; $\Sigma\omega$ -6 PUFA is the sum of 18:2 ω 6, 18:3 ω 6, 20:4 ω 6, 20:3 ω 6, 20:2 ω 6, 22:5 ω 6, 22:4 ω 6.

^c FA not found (%total FA = 0) were 20:4 ω 3, 20:2 ω 6.

Kidney: The 18:1 ω 9 (oleic acid) composition of the kidney was higher for the low-supplementation group compared to the control, medium, and high-supplementation groups (P<0.05) (Table 7). The 22:5 ω 6 (docosapentaenoic acid) composition of the kidney for the medium and high supplementation was highest at 0.9% and 0.5%, respectively, which significantly differed (P<0.05), whereas the other compositions

did not (Table 7). The 22:6 ω 3 (docosahexaenoic acid) composition of the kidney for the high supplementation was the highest at 3.4% with a significant difference ($P < 0.05$) from the control, low, and medium supplementation levels (Table 7). The Σ PUFA kidney composition of the medium and high supplementation levels was higher compared to the control and low levels of supplementation ($P < 0.05$) (Table 7).

Liver: The liver from the control and low-supplementation groups had the highest 14:0 (myristic acid) compositions of 0.6% and 0.5%, respectively, with significance ($P < 0.01$), followed by the lowest observed 14:0 composition of 0.3% in the medium and high-supplementation groups (Table 8). The 20:5 ω 3 (eicosapentaenoic acid) composition of the liver from the low, medium, and high-supplementation groups were significantly higher ($P < 0.05$) than the control supplement level of 1.0% (Table 8). For 20:3 ω 6 (dihomo- γ -linolenic acid) in the liver, the highest compositions were found for both the medium and high supplementation levels at 0.6% and 0.5%, respectively (Table 8). The composition of 22:5 ω 6 (docosapentaenoic acid) in the liver was the highest at 1.1% for the medium-supplementation group, which significantly differed from the other supplementation levels ($P < 0.05$) (Table 8).

Discussion

Spirulina has been the subject of recent studies in sheep (Kashani et al., 2015; Holman et al., 2014 a, 2014 b, 2014 c; Holman and Malau-Aduli, 2014; Holman and Malau-Aduli, 2013; Holman et al., 2012), but there is still a knowledge gap with regard to its impact on FA composition. In this study, FA data were converted into percentages of total FA composition (as % total FA). Aspects of the FA profile were evaluated to provide deeper insight into the effect of *Spirulina* supplementation on FA composition in Australian dual-purpose lambs. It was demonstrated that the medium-level *Spirulina* diet resulted in higher polyunsaturated fatty acid (PUFA) compositions.

Studies have demonstrated that diets with high FA content have a major impact on the fatty acid composition of ruminants (Alfaia et al., 2009; Raes et al., 2004; Wachira et al., 2007). Research has shown that ruminant PUFA including ω -3 and ω -6 content, can be improved by increased dietary intake of α -linolenic acid (ALA) and linoleic acid (LA) (Wachira et al., 2007; Wood and Enser, 1997; Wood et al., 2004). ALA is an important ω -3 fatty acid that is used as a precursor for the production of long chain ω -3 fatty acids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Abeywardena and Patten, 2011; Belay et al., 1993; Daley et al., 2010; Kouba and Mouro, 2011; Raes et al., 2004). Similarly, LA is the most prevalent ω -6 fatty acid that is converted to its longer chain counterpart arachidonic acid (ARA) through a series of conversions (Doreau et al., 2010; Price et al., 2000; Woods and Fearon, 2009).

The *Spirulina* diet was rich in the essential fatty acids ALA, LA, and DGLA (Iwata et al., 1990; Qureshi et al., 1996; Ross and Dominy, 1990). Thus, it is acceptable to presume that these dietary ingredients contributed to the observed levels of individual and total tissue ω -3 and ω -6 PUFAs (Fokkema et al., 2002; Pethick et al., 2010). In our study, the medium-level *Spirulina* diet increased EPA and LA significantly in subcutaneous adipose tissue, which increased the percentage of $\Sigma\omega$ -6 PUFA. Research has demonstrated that EPA is an essential and physiologically significant ω -3 fatty acid (Abeywardena and Patten, 2011; Belay et al., 1993; Daley et al., 2010; Mapiye et al., 2011; Woods and Fearon, 2009) because it is an essential ω -3 fatty acid that is needed by the human body, which cannot synthesise EPA and must thus acquire it from food or supplement sources.

It was discovered that the medium *Spirulina* diet increased EPA in subcutaneous adipose, muscle, and liver tissues. The low *Spirulina* diet reduced the percentage of pentadecanoic acid in the heart tissue (Hoashi et al., 2008; Scollan et al., 2001) compared to the adipose tissue. This is because the *Spirulina* supplement is rich in α -linolenic acid. Pentadecanoic acid is a saturated FA found in milk fat from cows (Cooper et al., 2004; DeBusk, 2010), and it is also detected in hydrogenated mutton fat (Scollan et al., 2001; Woods and Fearon, 2009). This finding suggests that the low-level *Spirulina* diet reduces the conversion of unsaturated fatty acids to saturated fatty acids thus supporting the theory of reduced biohydrogenation (Kouba and Mouro, 2011; Pethick et al., 2010) in crossbred lambs. An increase in eicosadienoic acid (20:2 ω -6) was observed in the tissues associated with the low-level *Spirulina* diet.

Eicosadienoic acid (EDA) is a naturally occurring ω -6 PUFA found mainly in animal tissues (Daley et al., 2010). EDA is elongated from linoleic acid (LA) (Kouba and Mouro, 2011; Price et al., 2000) and can be further metabolised to dihomo- γ -linolenic acid (DGLA) and arachidonic acid (AA) (Daley et al., 2010; Mapiye et al., 2011; Price et al., 2000). This indicates that an LA-rich diet can increase EDA elongation, which is then further metabolized to arachidonic acid (Mapiye et al., 2011; Moibi and Christopherson, 2001; Nguyen et al., 2010). The role of arachidonic acid includes keeping cell membranes flexible and permeable (Pethick et al., 2010; Price et al., 2000), and it also promotes muscle growth (Fokkema et al., 2002; Rowe, 2010; Santos-Silva et al., 2002).

It was demonstrated that the medium *Spirulina* diet significantly affected the percentage of docosapentaenoic acid in kidney tissue. Docosapentaenoic acid is an ω -3 FA (Kouba and Mouro, 2011). It is formed metabolically from linolenic acid and is a constituent of animal glycerophospholipids (Scollan et al., 2001; Smet et al., 2004; Wachira et al., 2007). This highlights that a *Spirulina* diet rich in LA increases other ω -6 PUFAs in Australian crossbred lambs, including long-chain ω -6.

The *Spirulina* diets were shown to affect the composition of oleic acid in *Longissimus dorsi* muscle tissue. Oleic acid is classified as a monounsaturated ω -9 FA (Daley et al., 2010; Kouba and Mouro, 2011). Previous studies have found significant variation of FA composition in response to dietary supplementation (Alfaia et al., 2009; Doreau et al., 2010; Mapiye et al., 2011; Scollan et al., 2001). The biosynthesis of oleic acid involves the action of the enzyme stearoyl-CoA 9-desaturase (Wood et

al., 2008) acting on stearoyl-CoA (Price et al., 2000; Woods and Fearon, 2009) and the dehydrogenation of stearic acid to give the MUFA derivative oleic acid (Wood et al., 2008; Wood et al., 2004; Woods and Fearon, 2009).

The *Spirulina* diet resulted in the reduction of palmitic acid (C16:0), which is the first fatty acid produced during fatty acid synthesis (Wood et al., 2008; Wood et al., 2004). Palmitic acid is a precursor to longer fatty acids (Wachira et al., 2002) that is regulated by a negative feedback controlled by acetyl-CoA carboxylase (ACC) (Kouba and Mourou, 2011; Santos-Silva et al., 2002). ACC is responsible for converting acetyl-CoA to malonyl-CoA (Smet et al., 2004; Wood and Enser, 1997), which is used to add to the growing acyl chain, thus preventing further palmitate generation (Price et al., 2000; Raes et al., 2004; Rowe, 2010). Research has demonstrated that palmitic acid increases the risk of developing cardiovascular diseases, thus lower levels are favourable for human health (Abeywardena and Patten, 2011; Belay et al., 1993; Fokkema et al., 2002). In this study, the compositions of *longissimus* and heart muscles were favourably improved in lambs supplemented with medium levels of *Spirulina* as this treatment produced the lowest levels of palmitic acid and highest levels of linoleic acid and $\Sigma\omega$ -6 PUFA.

Conclusion

This study has identified the composition variation in the FA profiles of lamb adipose, muscle, heart, kidney and liver tissues attributable to differences in the level of *Spirulina* supplementation. The medium level of *Spirulina* supplementation elevated the proportions of ω -3 and ω -6 PUFA in all tissues and organs. Low and high levels of *Spirulina* supplementation slightly increased PUFA in some tissues and organs. Based on this study's findings, the use of 100 mL/head/day of *Spirulina* supplementation is optimal for enhancing PUFA levels in lamb from dual-purpose sheep production systems utilising ryegrass pastures as basal diet.

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References

- Abeywardena M.Y., Patten G.S. (2011). Role of omega-3 long-chain polyunsaturated fatty acids in reducing cardio-metabolic risk factors. *Endocr. Metab. Immun. Disord. Drug Targets*, 22: 223–235.
- Afolayan R.A., Adayinka I.A., Lakpini C.A.M. (2006). The estimation of live weight from body measurements in Yankasa sheep. *Czech J. Anim. Sci.*, 51: 343–348.
- Alfaia C.P.M., Alves S.P., Martins S.I.V., Costa A.S.H., Fontes C.M.G.A., Lemos J.P.C., Bessa R.J.B., Prates J.A.M. (2009). Effect of the feeding system on intramuscular fatty acids and conjugated linoleic acid isomers of beef cattle, with emphasis on their nutritional value and discriminatory ability. *Food Chem.*, 114: 939–946.

- Belay A., Ota Y., Miyakawa K., Shimamatsu H. (1993). Current knowledge on potential health benefits of *Spirulina*. *J. Appl. Phycol.*, 5: 235–241.
- Bligh E.G., Dyer W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian J. Biochem. Physiol.*, 37: 911–917.
- Cooper S.L., Sinclair L.A., Wilkinson R.G., Hallett K.G., Enser M., Wood J.D. (2004). Manipulation of the ω -3 polyunsaturated fatty acid content of muscle and adipose tissue in lambs. *J. Anim. Sci.*, 82: 1461–1470.
- Daley C.A., Abbott A., Doyle P.S., Nader G.A., Larson S. (2010). A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutr. J.*, 9:10; doi:10.1186/1475-2891-9-10.
- DeBusk R. (2010). The role of nutritional genomics in developing an optimal diet for humans. *Nutr. Clin. Prac.*, 25: 627–633.
- Doreau M., Bauchart D., Chilliard Y. (2010). Enhancing fatty acid composition of milk and meat through animal feeding. *Anim. Prod. Sci.*, 51: 19–29.
- Fokkema M.R., Smit E.N., Martini I.A., Woltil H.A., Boersma E.R., Muskiet F.A.J. (2002). Assessment of essential fatty acid and ω 3-fatty acid status by measurement of erythrocyte 20:3 ω 9 (Mead acid), 22:5 ω 6/20:4 ω 6 and 22:5 ω 6/22:6 ω 3. *Prostagland. Leukotri. Essen. Fatty Acids*, 67: 345–356.
- Hoashi S., Hinenoya T., Tanaka A., Ohsaki H., Sasazaki S., Taniguchi M., Oyama K., Mukai F., Mannen H. (2008). Association between fatty acid compositions and genotypes of FABP4 and LXR-alpha in Japanese Black cattle. *BMC Genet.*, 9, p. 84; doi:10.1186/1471-2156-9-84; <http://www.biomedcentral.com/1471-2156/9/84>
- Holman B.W.B., Kashani A., Malau-Aduli A.E.O. (2014 a). Effects of *Spirulina* (*Arthrospira latensis*) supplementation level and basal diet on liveweight, body conformation and growth traits in genetically divergent Australian dual-purpose lambs during simulated drought and typical pasture grazing. *Small Rum. Res.*, 120: 6–14; doi <http://dx.doi.org/10.1016/j.smallrumres.2014.04.014>
- Holman B.W.B., Kashani A., Malau-Aduli A.E.O. (2014 b). Wool quality traits of purebred and crossbred Merino lambs orally drenched with *Spirulina* (*Arthrospira platensis*). *Italian J. Anim. Sci.*, 13: 387–391; doi: 10.4081/ijas.2014.3174.
- Holman B.W.B., Flakemore A.R., Kashani A., Malau-Aduli A.E.O. (2014 c). *Spirulina* supplementation, sire breed, sex and basal diet effects on lamb intramuscular fat percentage and fat melting points. *Int. J. Vet. Med. Res. Rep.* 2014, Article ID 263951; doi: 10.5171/2014.263951, 9 pp.
- Holman B.W.B., Malau-Aduli A.E.O. (2014). Effects of *Spirulina* supplementation on wool quality in purebred and crossbred Merino lambs fed pasture and lucerne hay basal diets. *J. Agric. Sci.* 6: 120-127; doi: 10.5539/jas.v6n7p120.
- Holman B.W.B., Malau-Aduli A.E.O. (2013). *Spirulina* as a livestock supplement and animal feed. *J. Anim. Physiol. Anim. Nutr.*, 97: 615–623; doi: 10.1111/j.1439-0396.2012.01328.x.
- Holman B.W.B., Kashani A., Malau-Aduli A.E.O. (2012). Growth and body conformation responses of genetically divergent Australian sheep to *Spirulina* (*Arthrospira platensis*) supplementation. *American J. Expt. Agric.*, 2: 160–173.
- Iwata K., Inayama T., Kato T. (1990). Effects of *Spirulina platensis* on plasma lipoprotein lipase activity in fructose-induced hyperlipidemic rats. *J. Nutri. Sci. Vit.*, 36: 165–171.
- Kashani A., Holman B.W.B., Nichols P.D., Malau-Aduli A.E.O. (2015). Effect of dietary supplementation with *Spirulina* on the expression of *AANAT*, *ADRB3*, *BTG2* and *FASN* genes in the subcutaneous adipose and *Longissimus dorsi* muscle tissues of purebred and crossbred Australian sheep. *J. Anim. Sci. Tech.*, 57, p. 8; doi:10.1186/s40781-015-0047-3.
- Kouba M., Mourot J. (2011). A review of nutritional effects on fat composition of animal products with special emphasis on ω -3 polyunsaturated fatty acids. *Biochimie*, 93: 13–17.
- Mapiye C., Chimonyo M., Dzama K., Hugo A., Strydom P.E., Muchenje V. (2011). Fatty acid composition of beef from Nguni steers supplemented with *Acacia karroo* leaf-meal. *J. Food Comp. Anal.*, 34: 556–567.
- McLeod B.M., White A.K., O'Halloran W.J. (2010). Marketing of sheep and sheep meat. In: *International Sheep and Wool Handbook*, D.J. Cottle (Ed.), Nottingham University Press: Nottingham, UK, pp. 677–690.

- Miller M.R., Nichols P.D., Barnes J., Davies N.W., Peacock E.J., Carter C.G. (2006). Regiospecificity profiles of storage and membrane lipids from the gill and muscle tissue of Atlantic salmon (*Salmo salar* L.) grown at elevated temperature. *Lipids*, 41: 865–876.
- Moibi J.A., Christopherson R.J. (2001). Effect of environmental temperature and a protected lipid supplement on the fatty acid profile of ovine *longissimus dorsi* muscle, liver and adipose tissues. *Livest. Prod. Sci.*, 69: 245–254.
- Nguyen N.H., Ponzoni R.W., Yee H.Y., Abu-Bakar K.R., Hamzah A., Khaw H.L. (2010). Quantitative genetic basis of fatty acid composition in the GIFT strain of Nile tilapia (*Oreochromis niloticus*) selected for high growth. *Aquaculture*, 309: 66–74.
- Pethick D.W., Ball A.J., Banks R.G., Hocquette J.F. (2010). Current and future issues facing red meat quality in a competitive market and how to manage continuous improvement. *Anim. Prod. Sci.*, 51: 13–18.
- Phythian C.J., Hughes D., Michalopoulou E., Cripps P.J., Duncan J.S. (2012). Reliability of body condition scoring of sheep for cross-farm assessments. *Small Rum. Res.*, 104: 156–162.
- Price P.T., Nelson C.M., Clarke S.D. (2000). Omega-3 polyunsaturated fatty acid regulation of gene expression. *Current Opin. Lipidol.*, 11: 3–7.
- Qureshi M.A., Garlich J.D., Kidd M.T. (1996). Dietary *Spirulina platensis* enhances humoral and cell-mediated immune functions in chickens. *Immunopharmacol. Immunotoxicol.*, 18: 465–476.
- Raes K., De Smet S., Demeyer D. (2004). Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: A review. *Anim. Feed Sci. Tech.*, 113: 199–221.
- Ross E., Dominy W. (1990). The nutritional value of dehydrated, blue-green algae (*Spirulina platensis*) for poultry. *Poultry Sci.*, 69: 794–800.
- Rowe J.B. (2010). The Australian sheep industry – undergoing transformation. *Anim. Prod. Sci.*, 50: 991–997.
- Santos-Silva J., Bessa R.J.B., Santos-Silva F. (2002). Effect of genotype, feeding system and slaughter weight on the quality of light lambs: II. Fatty acid composition of meat. *Livest. Prod. Sci.*, 77: 187–194.
- Scollan N.D., Choi N.J., Kurt E., Fisher A.V., Enser M., Wood J.D. (2001). Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. *British J. Nutr.*, 85: 115–124.
- Smet S.D., Raes K., Demeyer D. (2004). Meat fatty acid composition as affected by fatness and genetic factors: a review. *Anim. Res.*, 53: 81–98.
- Sowande O.S., Sobola O.S. (2008). Body measurements of West African dwarf sheep as parameters for estimation of liveweight. *Trop. Anim. Hlth. Prod.*, 40: 433–439.
- Wachira A.M., Sinclair L.A., Wilkinson R.G., Enser M., Wood J.D., Fisher A.V. (2002). Effects of dietary fat source and breed on the carcass composition, ω -3 polyunsaturated fatty acid and conjugated linoleic acid content of sheep meat and adipose tissue. *Brit. J. Nutr.*, 88: 697–709.
- Wood J.D., Enser M. (1997). Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. *Brit. J. Nutr.*, 78 (Suppl. 1): S49–S60.
- Wood J.D., Enser M., Fisher A.V., Nute G.R., Sheard P.R., Richardson R.I., Hughes S.I., Whittington F.M. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Sci.*, 78: 343–358.
- Wood J.D., Richardson R.I., Nute G.R., Fisher A.V., Campo M.M., Kasapidou E., Sheard P.R., Enser M. (2004). Effects of fatty acids on meat quality: a review. *Meat Sci.*, 66: 21–32.
- Woods V.B., Fearon A.M. (2009). Dietary sources of unsaturated fatty acids for animals and their transfer into meat, milk and eggs: A review. *Livest. Sci.*, 126: 1–20.

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