


1 Article

## 2 Supplementing dairy ewes grazing low quality 3 pastures with plant-derived oils and rumen-protected 4 EPA+DHA pellets improves lactation traits and body 5 condition score

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21

22 **Simple Summary:** This study evaluated the lactation performance and body condition scores of  
23 purebred Awassi and Awassi x East Friesian crossbred dairy ewes grazing low quality pastures and  
24 supplemented with diverse plant-derived oil enriched pellets under on-farm management  
25 conditions. The results demonstrated that supplementation with rumen protected EPA+DHA and  
26 oil-infused pellets improved milk, fat and protein yields by approximately 30, 13, and 31%  
27 respectively, and crossbred ewes produced more milk than purebreds. These results are very useful  
28 for dairy sheep producers in improving ewe lactation performance, milk quality and body condition  
29 score under low quality pasture grazing conditions.

30 **Abstract:** The Australian dairy sheep industry is small and mostly based on a natural grass grazing  
31 system which can limit productivity. The current study tested different plant oil-infused and rumen  
32 protected polyunsaturated fats and their interactions with sire breeds to improve lactation traits and  
33 body condition score (BCS) of ewes grazing low quality pastures. It was hypothesised that  
34 *supplementing lactating ewe diets plant-derived polyunsaturated oils will improve milk production and*  
35 *composition without compromising BCS.* Sixty ewes (n=10/treatment) in mid-lactation, balanced by sire  
36 breed, parity, milk yield, body condition score, and liveweight were supplemented with: 1) control:  
37 wheat-based pellets without oil inclusion; wheat-based pellets including 2) canola oil (CO); 3) rice  
38 bran oil (RBO); 4) flaxseed oil (FSO), 5); safflower oil (SFO) and 6) rumen protected fat containing  
39 eicosapentaenoic acid and docosahexaenoic acid (RPO). Except for the control group, all  
40 supplementary diets included the same level of 50 ml/kg DM of oil and all diets were isocaloric and  
41 isonitrogenous. Experimental animals were grazed in the same paddock with *ad libitum* access to  
42 pasture, hay and water during the 10-week study. RPO was the most effective diet that enhanced  
43 milk, fat and protein yields by approximately 30, 13, and 31% respectively (P<0.0001). Significant  
44 increase in milk production was also observed in CO, RBO, and SFO (P<0.0001). Breed significantly  
45 influenced animal performance with higher milk yield recorded for crossbred Awassi x East Friesian  
46 (AW x EF) (578 g/day) vs purebred Awassi (452 g/day) (P<0.0001). This study provides empirical

47 evidence for the use of rumen-protected and plant-derived oil-infused pellets as supplements under  
48 low quality pasture grazing conditions, to improve production performance of purebred Awassi  
49 and crossbred AW x EF ewes.

50 **Keywords:** PUFA; oils; body condition score; sheep milk composition; supplementation; canola;  
51 flaxseed; safflower; rice bran;

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## 53 1. Introduction

54 Although previously published studies have demonstrated that sheep milk has more nutritional  
55 value compared to cow milk [1,2], the contribution of milk derived from sheep to national milk  
56 production in Australia is relatively low. As at 2013, there were 13 commercial farms producing  
57 550,000 litres of milk annually [3] compared to 9 billion litres of milk produced by dairy cows  
58 nationwide [4]. Milk yield and composition are influenced by various factors including diet, breed,  
59 age, management practices, health, and environment [5-7]. Dietary supplementation with fat is  
60 considered as an effective tool to improve milk yield and alter milk composition [8,9]. Plant derived  
61 oils are a potential source of dietary fat and have been used in ruminant feeds to increase the energy  
62 density of diets and modify milk fatty acid profile [7,10,11] with the aim of increasing n-3 long-chain  
63 ( $\geq$ C20) polyunsaturated fatty acids (n-3 LC-PUFA) in dairy products. This is because high  
64 consumption of n-3 LC-PUFA in humans has been demonstrated to inhibit adipogenic, diabetogenic,  
65 atherogenic [12], inflammatory [13,14] and carcinogenic [15] diseases and lower the risk of  
66 developing Alzheimer's disease [16]. A number of authors have demonstrated that while dietary fat  
67 supplements can enhance milk yield [17-20], it is accompanied by a decrease in milk fat and protein  
68 composition because of the negative correlation between milk solid concentration and milk yield  
69 [7,21]. This could reduce income of the producers as milk is generally traded based on total milk  
70 solids. For this reason, the use of fats as dietary sources to improve the milk yield of sheep used for  
71 commercial milk harvesting within Australia, is not widely undertaken and is mostly applied as a  
72 supplement only during the dry seasons when pasture quality and quantity are low, in order to  
73 increase the energy intake of lactating animals [22].

74 To our current knowledge, studies on the effect of dietary supplementation with rice bran,  
75 canola, and safflower oils on milk yield and composition have only been conducted with dairy cows  
76 [19,23,24] and goats [25], but not dairy ewes. The effects of supplementation with flaxseed on animal  
77 performance and milk fatty acid profiles have been studied with dairy ewes, however, these  
78 investigations supplemented flaxseed either as whole or extruded grain [26-28]. In addition, there  
79 has been a paucity of studies that have examined the effects of varying dietary supplementation on  
80 lactation and liveweight traits in grazing dairy ewes of different genetic backgrounds under the same  
81 management and feeding regime.

82 The major objective of the current work was to compare the lactation performance, milk  
83 composition and body condition score of dairy ewes in mid lactation grazing low quality pastures  
84 and supplemented with canola, rice bran, flaxseed, safflower and rumen protected oil-infused pellets.  
85 It was hypothesised that *supplementing grazing dairy ewes with oils of different plant-derived origins will*  
86 *have different effects on milk yield, milk composition and body condition score.*

## 88 2. Materials and Methods

### 89 *Animal ethics*

90 The use of animals and procedures performed in this study were all approved by the University  
91 of Tasmania Animal Ethics Committee (Permit No A0015657).

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97 2.1. *Animal management and experimental design*

98 Sixty lactating Awassi and crossbred Awassi x East Friesian ewes in mid-lactation, located in the  
 99 South East of Tasmania (Grandveve Cheeses Farm, Birchs Bay, Woodbridge, Tasmania, Australia)  
 100 were included in a ten-week feeding trial where the ewes were kept in the same paddock and had *ad*  
 101 *libitum* access to local natural velvet tussock grass, hay and water. The experimental animals were  
 102 allocated to six dietary treatments with each group balanced for liveweight, breed, parity, body  
 103 condition score (BCS), and milk yield. Treatments consisted of (1) commercial wheat-based pellets  
 104 without oil inclusion (control); wheat-based pellets infused with 50 ml/kg DM of (2) canola (CO); (3)  
 105 rice bran (RBO); (4) flaxseed (FSO); (5) safflower (SFO) and (6) rumen protected EPA+DHA (RPO)  
 106 oils represented in Table 1. All treatments were isocaloric and isonitrogenous (Table 2). Each ewe was  
 107 fed 1 kg/day of the supplemented pellet individually in the milking parlour during milking time over  
 108 a 10-week period with an initial two-week adjustment period, followed by an 8-week experimental  
 109 period. In the first two weeks of the adjustment period, commercial pellets (control), for each  
 110 treatment group were increasingly substituted at 100 g/day by experimental diets CO, RBO, FSO,  
 111 SFO, and RPO until the attainment of 1 kg/day on day 10 was achieved. Ewes were milked in the  
 112 mornings at 0600hrs and individual milk yield was electronically recorded by the La Laval platform  
 113 using De Laval's Alpro Herd Management System software (De Laval, The Netherlands).  
 114

115 **Table 1.** Ingredient composition of the experimental pellets<sup>a</sup>

Items	Control	CO	RBO	FSO	SFO	RPO
Ingredient, g/kg						
Wheat	585	545	535	465	535	530
Paddy rice	210	210	220	280	210	215
Lupins	148	148	148	148	148	148
Canola oil, ml/kg	-	50	-	-	-	-
Flaxseed oil, ml/kg	-	-	-	50	-	-
Safflower oil, ml/kg	-	-	-	-	50	-
Rice bran oil, ml/kg	-	-	50	-	-	-
EPA+DHA, ml/kg	-	-	-	-	-	50
Ammonium sulphate	12.6	12.6	12.6	12.6	12.6	12.6
Salt	10	10	10	10	10	10
Limestone	20.9	20.9	20.9	20.9	20.9	20.9
Sheep premix	1	1	1	1	1	1
Acid buff	6.25	6.25	6.25	6.25	6.25	6.25
Sodium bicarbonate	6.25	6.25	6.25	6.25	6.25	6.25

116 <sup>a</sup> Canola oil (CO), rice bran oil (RBO), flaxseed oil (FSO), safflower oil (SFO), rumen-protected oil (RPO).  
 117  
 118  
 119

120 **Table 2.** Nutrient compositions<sup>a</sup> of basal and experimental diets

Component (DM)	(% Pasture	Hay	Control	CO	RBO	FSO	SFO	RPO
DM	96.5	95.5	91.5	93.0	91.6	90.0	91.7	91.6
OM	90.5	97.3	92.2	93.3	92.7	91.0	91.8	92.0
Ash	9.5	2.7	7.8	6.7	7.3	9.0	8.2	8.0
ADF	45.5	37.6	10.6	7.1	8.1	9.7	9.0	8.5
NDF	69.9	68.3	30.0	21.8	19.4	23.3	23.9	22.0
EE	1.4	1.2	3.3	5.7	5.2	5.4	5.0	5.1
CP	4.7	4.3	14.6	14.0	14.7	14.6	14.5	15.6
TDN	48.5	54.1	73.4	75.9	75.2	74.1	74.5	74.9
ME, MJ/kg DM	7.1	8.1	11.7	12.2	12.0	11.8	11.9	12.0

121 <sup>a</sup> Dry matter (DM), organic matter (OM), acid detergent fibre (ADF), neutral detergent fibre (NDF), ether extract  
 122 (EE), crude protein (CP), total digestible nutrients (TDN) and metabolisable energy (ME).

123 All other abbreviations are as defined in Table 1.

124

#### 125 2.2. Feed intake and body condition score

126 The amount of offered pellets and residuals were weighed daily to calculate feed intake. Weekly  
 127 feed samples were collected and stored at -20°C for subsequent chemical analysis. Body condition  
 128 score (BCS) was subjectively evaluated at weekly intervals on a scale of 1-5 [29] by the same evaluator  
 129 to ensure consistency and repeatability.

130

#### 131 2.3. Milk sample analyses

132 Weekly milk samples from each animal were bulked from daily milkings at 0600hrs and stored  
 133 in labelled plastic vials containing a preservative at -20°C before sending the samples off to Hadspen  
 134 for compositional analysis at the officially contracted herd recording laboratory - TasHerd Pty Ltd,  
 135 Hadspen, Tasmania. The Fourier Transformed Infrared spectrometry technology (Bentley Fourier  
 136 Transform Spectrometer) was used to quantify milk composition. This system uses Bentley Flow  
 137 Cytometry to measure the somatic cell count, while the Bentley Fourier Transform Spectrometer  
 138 measures somatic cell count, milk fat, protein and lactose. The equation from Mavrogenis and  
 139 Papachristoforou [30] was used to calculate Fat-corrected milk (FCM):

140  $6\% \text{ FCM} = M (0.453 + 0.091F)$ , where “F” is the percentage of fat and “M” is milk yield (kg).

141

#### 142 2.4. Chemical analysis of experimental and basal diets

143 Before analysing dry matter (DM), ash and chemical composition, samples of the basal and  
 144 experimental diets were dried in a fan-forced oven at a constant temperature of 65°C and  
 145 subsequently ground through a 1 mm sieve using a Thomas Model 4 Laboratory Mill (Thomas  
 146 Scientific). DM content was determined by placing the ground samples at 150°C in an oven for 24 h  
 147 to remove moisture. The samples were combusted in a furnace set at 600°C for 8 h to determine ash  
 148 content. Neutral detergent fibre (NDF) and acid detergent fibre ADF were quantified using an  
 149 ANKOM220 fibre analyser, while an ANKOM<sup>XT15</sup> fat/oil extractor (ANKOM Technology Corp.,  
 150 Macedon, NY, USA) was used to measure ether extract. The crude protein percentage was calculated  
 151 based on the value of nitrogen that was determined using a Thermo Finnigan EA 1112 Series Flash  
 152 Elemental Analyser (Thermo Fisher Scientific, MA, USA). Table 2 shows the nutritional composition  
 153 of the experimental diets.

154

#### 155 2.5. Data and statistical analysis

156 All data were analysed using ‘Statistical Analysis System’ software [31]. Initial descriptive  
 157 summary statistics were computed with means, standard errors, and minimum and maximum values

158 scrutinised for data entry errors and outliers. The data were then subjected to General Linear Model  
 159 (PROC GLM) analysis, with different oil supplementation, sire breed, week of supplementation and  
 160 their interactions fitted as fixed effects and feed intake, milk yield, milk composition, and body  
 161 condition score as dependent variables. Level of significance threshold was  $P < 0.05$  and differences  
 162 between means were established using Duncan's multiple range and Turkey's probability pairwise  
 163 comparison tests. The final statistical model used for the analysis was:

$$164 Y_{ijk} = \mu + SB_i + D_j + W_k + (SBD)_{ij} + (SBW)_{ik} + (DW)_{jk} + e_{ijk}$$

165 Where  $Y_{ijk}$  is the dependent variable,  $\mu$  is the overall mean, SB, D and W are the fixed effects of  
 166 sire breed, diet and week of supplementation, respectively, brackets represent second-order  
 167 interactions and  $e_{ijk}$  is the error term.

168

### 169 3. Results

170 The results of this study suggest that dietary treatments significantly influenced feed intake of  
 171 grazing dairy ewes ( $P < 0.0001$ ; Table 3), with DM intakes being greatest in control group, followed  
 172 by the RBO, SFO, CO, RPO, and FSO groups respectively. Estimated intake of OM, ADF, NDF and  
 173 CP followed a similar pattern to DMI with the greatest intakes observed in the control group except  
 174 the intake of EE which was greatest in the RBO group (41 g/day). Breed and its interaction with  
 175 supplementation had no significant effect on intake (DMI), and were therefore excluded from Table  
 176 3.

177 **Table 3.** Least square means and standard errors (LSM  $\pm$ SEM) of experimental feed intake (g/head/day)

Items	Feed intake	DMI	OM	ADF	NDF	EE	CP
Treatment (T)							
Control	885.5 <sup>a</sup>	810.3 <sup>a</sup>	741.4 <sup>a</sup>	85.9 <sup>a</sup>	243.1 <sup>a</sup>	26.7 <sup>e</sup>	118.3 <sup>a</sup>
CO	751.3 <sup>c</sup>	698.7 <sup>c</sup>	651.9 <sup>b</sup>	49.6 <sup>e</sup>	152.3 <sup>d</sup>	39.8 <sup>b</sup>	97.8 <sup>e</sup>
RBO	860.4 <sup>b</sup>	788.0 <sup>b</sup>	730.5 <sup>a</sup>	63.8 <sup>c</sup>	152.9 <sup>d</sup>	40.9 <sup>a</sup>	115.8 <sup>b</sup>
FSO	754.3 <sup>c</sup>	678.9 <sup>d</sup>	617.8 <sup>d</sup>	65.9 <sup>b</sup>	158.2 <sup>c</sup>	36.7 <sup>c</sup>	99.1 <sup>e</sup>
SFO	767.1 <sup>c</sup>	703.4 <sup>c</sup>	645.8 <sup>bc</sup>	63.3 <sup>c</sup>	168.1 <sup>b</sup>	35.2 <sup>d</sup>	102.0 <sup>d</sup>
RPO	753.9 <sup>c</sup>	690.5 <sup>cd</sup>	635.3 <sup>c</sup>	58.7 <sup>d</sup>	151.9 <sup>d</sup>	35.2 <sup>d</sup>	107.7 <sup>c</sup>
Breed							
AW	793.5	726.5	678.8	64.3	170.6	35.7	106.5
AW x EF	797.1	729.9	671.9	64.7	171.5	35.8	107.0
SEM	4.1	3.8	3.5	0.6	1.7	0.3	0.6
P-values							
Treatment	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Breed	0.4483	0.4384	0.4423	0.3670	0.3492	0.5652	0.4358
T x Breed	0.7877	0.7982	0.7993	0.7557	0.6935	0.8934	0.8082

178 Dry matter intake (DMI).

179 Awassi (AW), East Friesian (EF), Awassi x East Friesian (AW x EF) crossbred.

180 All other abbreviations are as defined in Tables 1 and 2.

181 Values with different superscripts within columns are significantly different ( $P < 0.05$ ).

182

183 **Table 4.** Effect of supplementation with diverse plant-derived oils on body condition score and lactation  
184 performance traits

Item	MY	FCM	Fat	FY	Protein	PY	Lacto-se	SNF	SCC	BCS
Treatment (T)										
Control	484 <sup>d</sup>	542 <sup>bc</sup>	7.4 <sup>a</sup>	36 <sup>bc</sup>	5.4 <sup>c</sup>	26 <sup>c</sup>	4.9	10.9 <sup>bc</sup>	109 <sup>a</sup>	2.1 <sup>c</sup>
CO	525 <sup>c</sup>	573 <sup>b</sup>	7.2 <sup>ab</sup>	38 <sup>b</sup>	5.5 <sup>bc</sup>	29 <sup>b</sup>	4.9	11.1 <sup>bc</sup>	98 <sup>ab</sup>	2.3 <sup>a</sup>
RBO	527 <sup>c</sup>	578 <sup>b</sup>	7.2 <sup>ab</sup>	38 <sup>b</sup>	5.9 <sup>a</sup>	31 <sup>b</sup>	4.9	11.7 <sup>a</sup>	73 <sup>c</sup>	2.2 <sup>bc</sup>
FSO	489 <sup>d</sup>	523 <sup>c</sup>	6.9 <sup>bc</sup>	34 <sup>c</sup>	5.4 <sup>c</sup>	26 <sup>c</sup>	4.8	10.8 <sup>c</sup>	60 <sup>c</sup>	2.3 <sup>a</sup>
SFO	562 <sup>b</sup>	587 <sup>b</sup>	6.6 <sup>c</sup>	37 <sup>b</sup>	5.6 <sup>b</sup>	31 <sup>ab</sup>	4.8	11.2 <sup>b</sup>	105 <sup>ab</sup>	2.2 <sup>bc</sup>
RPO	628 <sup>a</sup>	649 <sup>a</sup>	6.6 <sup>c</sup>	41 <sup>a</sup>	5.4 <sup>c</sup>	34 <sup>a</sup>	4.8	11.0 <sup>bc</sup>	81 <sup>bc</sup>	2.2 <sup>bc</sup>
Breed (B)										
AW	496 <sup>b</sup>	535 <sup>b</sup>	7.1	35 <sup>b</sup>	5.5	27 <sup>b</sup>	4.8 <sup>b</sup>	11.1	97 <sup>a</sup>	2.2 <sup>b</sup>
AW × EF	578 <sup>a</sup>	617 <sup>a</sup>	6.9	40 <sup>a</sup>	5.5	32 <sup>a</sup>	4.9 <sup>a</sup>	11.2	78 <sup>b</sup>	2.3 <sup>a</sup>
SEM	3.4	7.8	0.07	3.6	0.04	2.9	0.02	0.05	3.6	0.0
P-values										
Treatment	0.0001	0.0001	0.0001	0.0021	0.0001	0.0001	0.1689	0.0001	0.0002	0.0018
Breed (B)	0.0001	0.0001	0.1765	0.0001	0.7444	0.0001	0.0006	0.1351	0.115	0.0030
Week (W)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0257	0.0012	0.0001
T × B	0.0001	0.0001	0.0001	0.0002	0.0003	0.0001	0.0001	0.0257	0.0795	0.0002
T × W	1.0000	1.0000	0.9766	0.9999	0.8717	1.0000	0.8348	0.8039	0.3630	0.9999
B × W	0.9061	0.8724	0.9494	0.8517	0.9971	0.9380	0.6808	0.9910	0.9974	0.8640

185 Milk yield (MY, g/day), fat-corrected milk (FCM, g/day), fat (g/100g milk), fat yield (FY, g/day), protein (g/100g  
186 milk), protein yield (PY, g/day), solids-non-fat (SNF), somatic cell count (SCC, ×1000 cells/ml), body condition  
187 score (BCS).

188 All other abbreviations are as defined in Table 1, 2.

189 Values with different superscripts within columns are significantly different ( $P < 0.05$ ).

190



191 Significant differences in dairy performance traits, milk composition, and body condition score  
192 were observed across treatments (Table 4). Ewes receiving RPO produced the greatest milk yield at  
193 628 g/day, followed by SFO, RBO, CO, FSO, and the control ( $P<0.0001$ ). Inconsistent with milk  
194 yield, fat concentration was highest in milk from control ( $P=0.015$ ), whereas RBO yielded the  
195 greatest content of protein (5.9 g/100g) ( $P<0.0001$ ). Although milk from ewes fed RPO had the least  
196 proportion of fat and protein at 6.6 and 5.4 (g/100g), respectively, this group produced the greatest  
197 fat yield (FY) (41 g/day;  $P=0.0008$ ) and protein yield (34 g/day;  $P=0.0004$ ). There were no significant  
198 differences among treatments in the percentage of milk lactose. The type of oil included in the  
199 dietary supplement affected body conformation ( $P=0.0008$ ), although the mean BCS of experimental  
200 ewes only varied from 2.1-2.3 (Table 4).

201 Weekly trends for BCS and lactation traits are presented in Figures 1 and 2. As observed in all  
202 treatment groups, BCS, fat percentage and protein percentage (Figure 1a, 2a, and 2b) increased,  
203 while milk yield decreased over the duration of the experimental period (Figure 1b). The best  
204 weekly milk yield trend was recorded in RPO group, where its decrease was smaller (4.9 at the start  
205 to 3.9 kg/week) than the other groups at the end of the trial.

206 Figure 3 presents significant interactions between oil supplementation and breed in milk yield  
207 ( $P<0.0001$ ), fat percentage ( $P<0.0001$ ), and protein percentage ( $P=0.0262$ ). Regarding milk  
208 production, crossbred AW x EF ewes had greater responses to oil supplements than AW with the  
209 highest milk yield at 751 g/day observed in RPO group (Figure 3a). Breed and diet interactions,  
210 however, were varied across treatments in which AW ewes fed with RBO produced the highest  
211 percentages of fat and protein (7.8, and 6.1 g/100g, respectively).

212

213

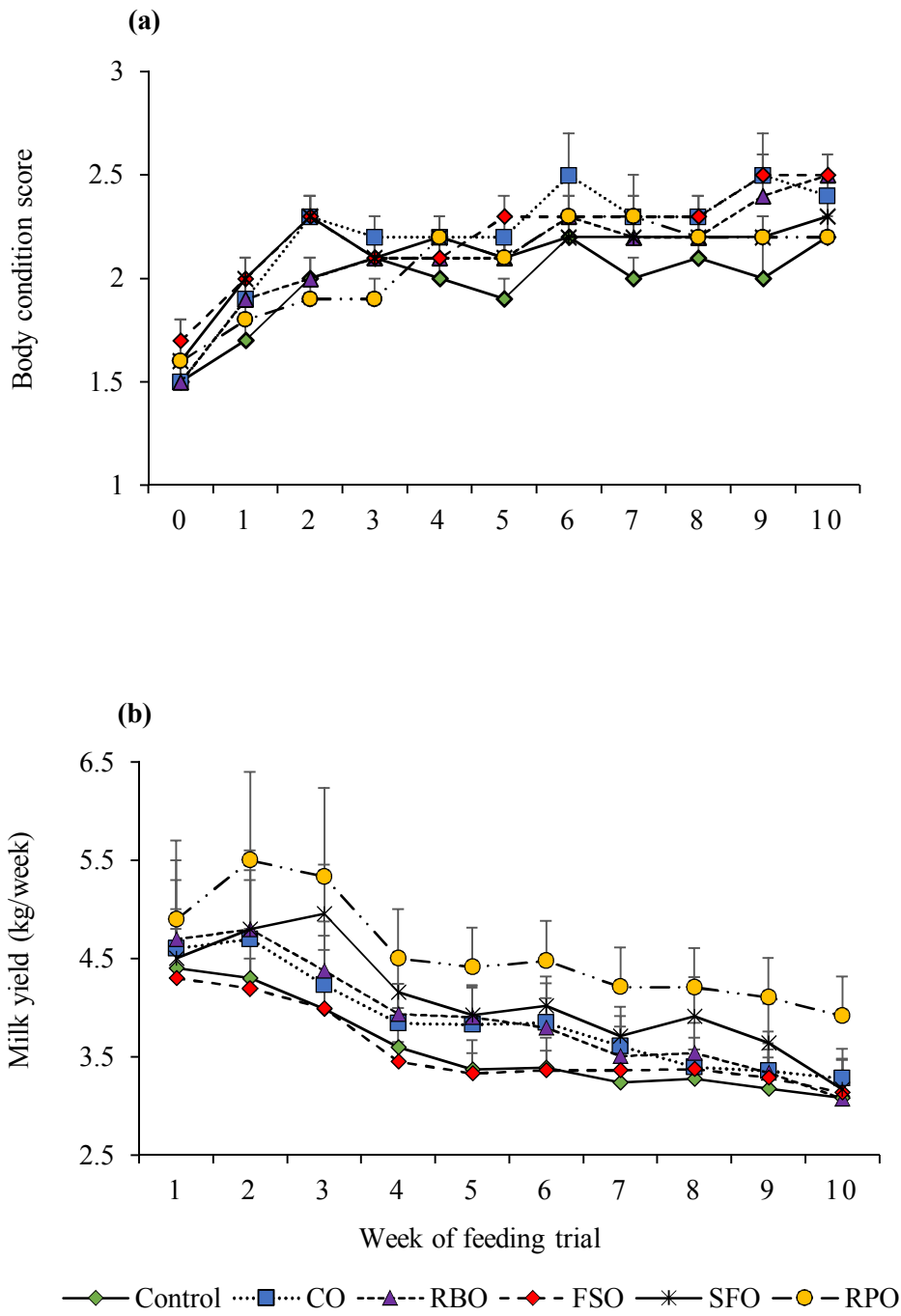
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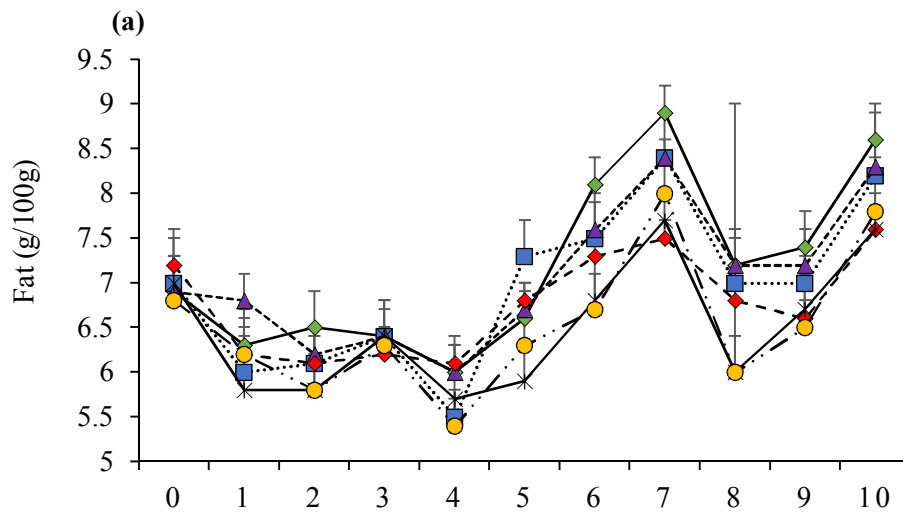
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Figure 1. Weekly trends in body condition score (a) and milk yield (b)

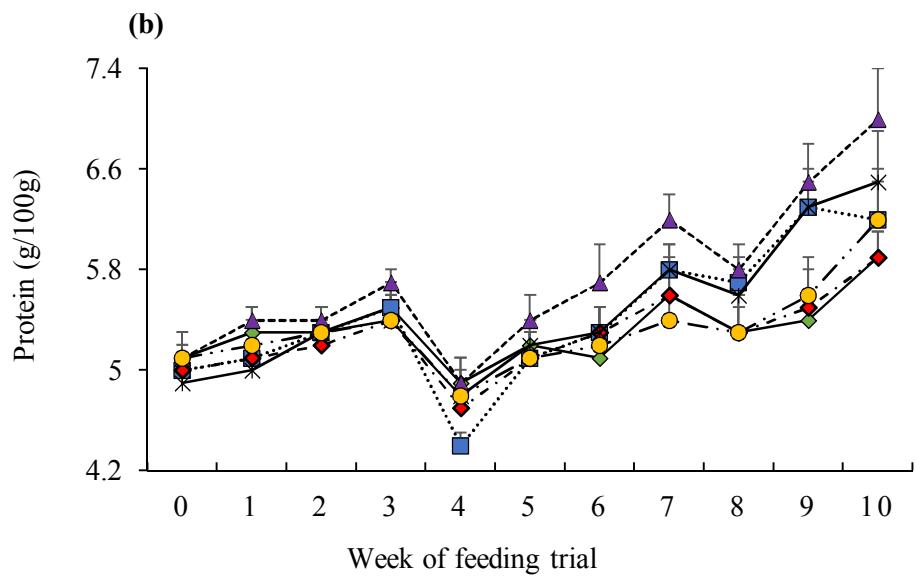


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231



—◆— Control    ···■··· CO    - -▲- - RBO    - -◆- - FSO    —\*— SFO    —●— RPO

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**Figure 2.** Weekly trends in milk fat (a) and milk protein (b) concentration

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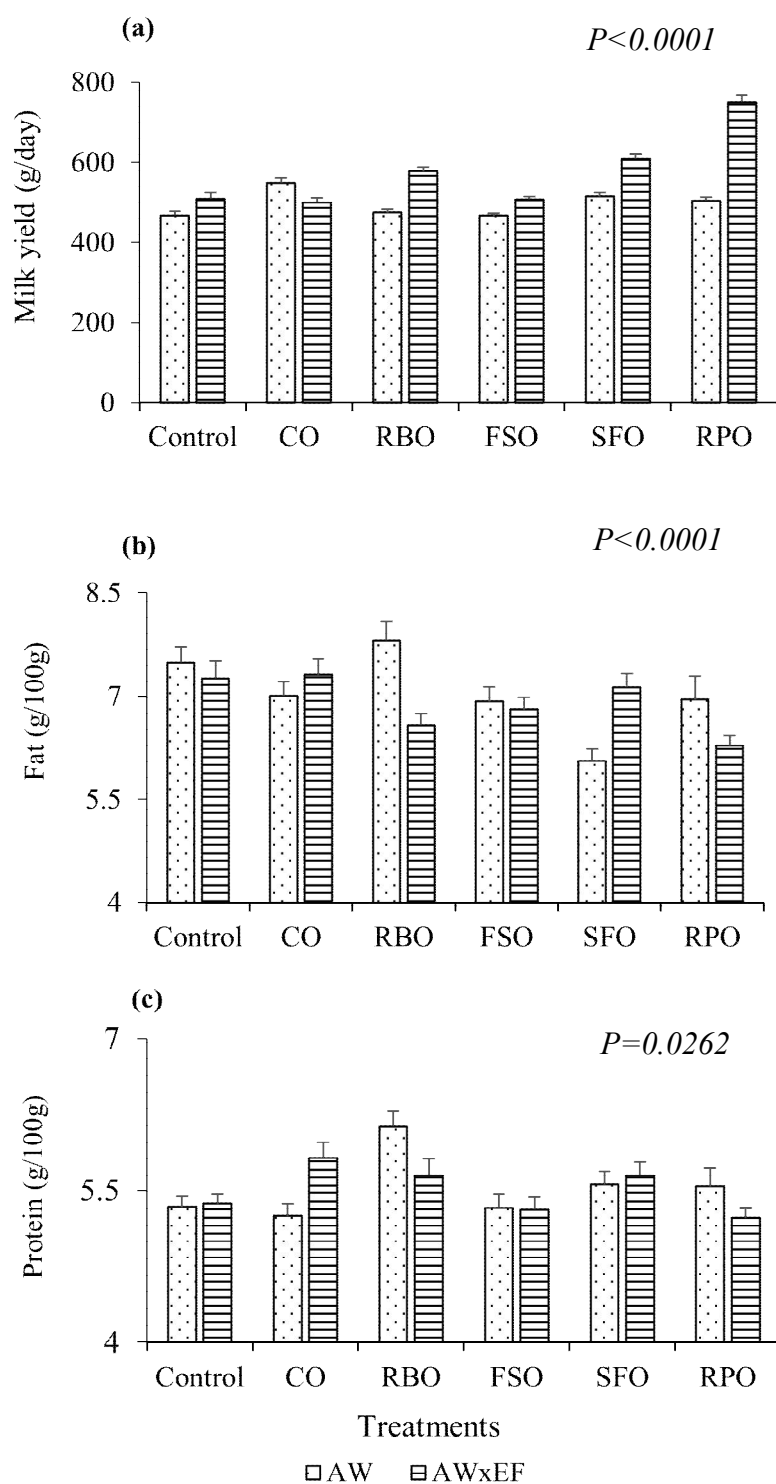


Figure 3. Supplementary diet and breed interactions on (a) milk yield, (b) milk fat, and (c) milk protein

## 266 4. Discussion

### 267 4.1 *Effect of dietary supplements on dry matter intake and body condition score*

268 The decrease in DMI was inconsistent with previous studies that examined the effect of adding  
269 2% plant oil in the diets of dairy ewes [32], but was similar to recent reports in dairy cows that found  
270 a negative impact of a high level supplemented oil on DMI [24,33-35]. According to Illius et al. [36]  
271 voluntary ruminant feed intake is affected by nutrient and energy flows related to ruminal  
272 fermentation. Adding high levels of oil in diets that was the case of the current study, may reduce  
273 diet acceptability [37] which is caused by ruminal function reduction. Other studies have shown that  
274 oil addition to diets reduces fibre digestibility, DMI and feed palatability in ruminants, suggesting  
275 negative effects of plant oils on animals' appetite. This occurs due to selection against microorganisms  
276 with cellulolytic capability leading to a decrease in ruminal fibre digestion [38]. Moreover, DMI  
277 differences among oil supplement groups (with the highest observed in RBO), indicates the effect of  
278 oil type on nutrient digestibility [39].

279 Known as an important indicator of cow heath status in dairy management, body condition  
280 score (BCS) is also regularly used to estimate fatness in the form of energy reserves as well as animal  
281 welfare status [40-43]. A meta-analysis by Kenyon et al. [29] demonstrated a positive association  
282 between BCS at breeding and ewe reproductive traits (pregnancy rate and number of lambs born).  
283 Generally, these parameters increase as BCS increases from 2.0 to 3.0 [44-46]. At the commencement  
284 of the feeding trial, the average BCS of the experimental animals was 1.5; a reflection of the low  
285 quality pastures the ewes were grazing and a pointer to fat mobilisation from body reserves for  
286 sustaining milk synthesis [47]. At the end of the feeding trial, average BCS values of ewes fed CO,  
287 RBO and FSO rose to 2.55, 2.60, and 2.55, respectively. These BCS were within the target of 2.5-3.0  
288 [29], which suggests that the use of such supplements could have a positive effect on not only milk  
289 yield, but also reproductive performance and the general welfare of dairy ewes.

### 291 4.2 *Effect of dietary supplements on milk yield, and milk composition*

292 Despite the wide accessibility and availability of canola and rice bran in Australia [48,49], the  
293 extent of use of these plant lipid sources as dietary supplements in the Australian dairy industry is  
294 unknown. Supplementing diets with canola and rice bran oils in the current study increased milk  
295 yield without exerting negative effects on milk fat and protein compositions. Lunsin et al. [24]  
296 supplemented dairy cow diets with 2, 4, 6% rice bran oil in a confined system and did not observe  
297 any statistical variation in milk production. This was inconsistent with a reduction in the milk yield  
298 of dairy goats fed total mixed rations that included 5, 10 and 20% rice bran [25]. In contrast, an  
299 increase in milk yield of RBO group observed in the current study suggests the advanced effect of  
300 rice bran oil inclusion in a pasture-based system compared to a confined system. Regarding milk fat  
301 and protein concentrations, supplementation of grazing dairy ewes with rice bran oil in the current  
302 study, had no influence on milk fat. However, it significantly enhanced milk protein even though the  
303 potential to alter milk protein concentration by changing the dietary composition is considered less  
304 compared with the potential to alter milk fat composition [9]. This increment of change in protein  
305 composition in milk agrees with the findings of Park et al. [25] in goat milk, but disagrees with a  
306 decrease observed in cows when the percentage of dietary RBO increased [24]. On the other hand,  
307 supplementation of ewes in this study and cows [19] in similar pasture-based dairy systems with 5%  
308 of CO demonstrated an increase in milk yield. However, while inclusion of CO had no statistically  
309 significant effect on all milk components of lactating ewes, Otto et al. [19] reported marginal decreases  
310 in fat and protein percentages of cow milk. These contrasting results in response to rice bran and  
311 canola oil supplementation suggest that there could be physiological differences between species in  
312 lipid metabolisms that might need further investigation.

313 Variation in results assessing the effect of whole or extruded flaxseed, but not flaxseed oil on  
314 milk production and composition of dairy ewes, have been reported [50]. Akin to the current results,  
315 no statistical difference in milk production was observed when ewes were supplemented with  
316 extruded linseed at 128 g/day [51] and 220 g/day [52]. These findings were in contrast with other  
317 authors who distinguished either an increase [27] or a decrease [28] in milk yield of dairy ewes fed

318 250 g/day of whole flaxseed or 200 g/day of extrude flaxseed respectively. Milk fat depression in  
319 response to supplementation with FSO in this study was supported by other studies in sheep [51]  
320 and cows [33,53,54], but disagrees with others that showed no changes in sheep [27,52] or a minor  
321 increase in sheep [26,28], and goats [55]. These variations might be due to the multi nutritional effects  
322 including energy balance, NDF concentration, feed particle size that have strong correlations with  
323 milk yield and milk fat concentration [11].

324 Safflower, which is grown in over 60 countries [56], has been used widely as a supplement in  
325 ruminant diets [57]. Despite studies investigating the effects of using various types of safflower on  
326 bovine and caprine performance [58], there is relatively little information on its effectiveness as a  
327 supplement for influencing milk yield and composition in lactating ewes. In this study,  
328 supplementation of grazing dairy ewes with SFO increased milk production by 16%. This supports  
329 the findings of Ahmadpour et al. [59] who supplemented dairy cows with rolled safflower seed at 3  
330 and 6% and reported increases in milk yield by 2 and 9% respectively. Other studies have, however,  
331 reported no significant effects on milk yield when the diets of lactating cows [23,57,60,61] and goats  
332 [62] were supplemented with safflower oil or seed. Similarly, variable responses and changes in  
333 milk components had been observed when the diets of lactating does or cows were supplemented  
334 with safflower. Some results portrayed negative effects [23,33,62] which align with our results, while  
335 others did not observe any significant effects [57,59-61]. The wide range of inclusion rates and  
336 variation in dietary components in these studies might have led to the variable responses reported.

337 An outstanding enhancement of milk yield by approximately 30% compared to the control  
338 animals, was observed in ewes supplemented with RPO. Increases in fat (13%) and protein (31%)  
339 were also observed. These incremental improvements in milk yield and total solids production play  
340 an important role in positively enhancing the economic benefits for dairy sheep producers as most  
341 sheep milk is used for cheese making [63]. The quantity of cheese that can be produced from sheep  
342 milk is limited by the concentrations of fat and especially protein, in raw milk [11]. Reviews on bypass  
343 fat supplementation studies suggest a consistent increase in the milk production of lactating cows by  
344 5.5-24% [64], while variable responses were presented in lactating ewes [11]. According to Pulina et  
345 al. [11], positive effects of supplementing rumen-protected fat on dairy sheep production  
346 performance generally occur with feeding trials longer than 4 weeks. This was confirmed in the  
347 current work, while short-term studies had a minor reduction or no change [65-67]. In this study,  
348 we recorded a reduction in the concentration of milk fat in the RPO group. This agrees with the  
349 findings of Rotunno et al. [68] who fed ewes with 4 and 8% rumen-protected fat, whereas this  
350 disagreed with consistent increase in milk fat concentration reported by Pulina et al. [11]. Differences  
351 in dietary components, type and dosage of protected fat, feeding regimes, or stage of lactation might  
352 have accounted for this contrasting set of outcomes.

353

#### 354 4.3. *Effect of breed on animal performance*

355 The East Friesian (EF) breed of sheep was developed in northern Germany and the Netherlands,  
356 and has become one of the world's most productive dairy sheep. The EF has earned the reputation  
357 as the most productive dairy sheep breed in terms of milk yield [69]. However, it has a low ability to  
358 adapt under unfavourable environmental conditions, especially excessive heat and humidity [70].  
359 Thus, this breed has been used widely in crossbreeding systems to improve milk production of local  
360 breeds in various temperate environments [70-72]. Together with Awassi (AW), the predominant  
361 breed in The Eastern Mediterranean countries [73], EF was introduced to Australia in the 1990s, and  
362 since, has been used more widely in the dairy sheep industry as reported by the Australian Rural  
363 Industries Research and Development Corporation [74]. The improvement in milk yield without any  
364 negative effects on relative content of milk composition in crossbred ewes AW x EF was akin to  
365 Clement et al. [75], whereas it was inconsistent with Gootwine and Goot [70] who demonstrated  
366 similar milk volumes between AW and AW x EF. Local heat stress that leads to a depression of feed  
367 intake, milk production and reproduction [76,77], might be the principal factor for this performance  
368 variation by crossbreds in some studies. Moreover, statistically significant variation in the interaction  
369 between treatments and sire breed regarding milk production and composition, but not feed intake,

370 in the current research, suggests that gene regulation may be involved in experimental oil  
371 metabolism. Therefore, identification of regulated genes for milk yield and composition in response  
372 to plant and rumen-protected oil supplements needs to be investigated.

## 373 5. Conclusions

374 The current study demonstrated that canola, rice bran, safflower and rumen-protected  
375 EPA+DHA could improve lactation traits without any negative impact on BCS of dairy ewes grazing  
376 low quality pasture. Under the same nutrition and management conditions, crossbred AW × EF  
377 significantly showed greater lactation performance than AW. Utilising these oil supplements  
378 combined with crossbreeding the AW and EF sheep breeds, is therefore, recommended for Australian  
379 sheep milk producers utilising pasture-based systems. In addition, the novel potential of  
380 supplementing dairy sheep with rice bran and canola oils explored in this study, may need further  
381 research to better elucidate their metabolic mechanisms.

382  
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384 H.V.L., D.V.N.; J. R. O.; P. N.; software, A.E.O.M-A; validation, A.E.O.M-A, P.D.N. and B.S.M-A.; formal  
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399

## 400 References

- 401 1. Park, Y.W.; Juarez, M.; Ramos, M.; Haenlein, G.F.W. Physico-chemical characteristics of goat and sheep  
402 milk. *Small Ruminant Res.* **2007**, *68*, 88-113.
- 403 2. Silanikove, N.; Leitner, G.; Merin, U. The interrelationships between lactose intolerance and the modern  
404 dairy industry: Global perspectives in evolutionary and historical backgrounds. *Nutrients* **2015**, *7*, 7312-7331.
- 405 3. AgriFutures Australia. (2013). *Dairy Sheep* | *AgriFutures Australia*. [online] Available at:  
406 <https://www.agrifutures.com.au/farm-diversity/dairy-sheep/> [Accessed 31 Apr. 2018]
- 407 4. Dairy Australia (2018). *Milk*. [online] Available at:  
408 <https://www.dairyaustralia.com.au/industry/production-and-sales/milk> [Accessed 31 Apr. 2018].
- 409 5. Abd Allah, M.; Abass, S.; Allam, F.M. Factors affecting the milk yield and composition of Rahmani and  
410 Chios sheep. *International Journal of Livestock Production* **2011**, *2*, 24-30.
- 411 6. Ayadi, M.; Matar, A.; Aljumaah, R.; Alshaiikh, M.; Abouheif, M. Factors affecting milk yield, composition  
412 and udder health of Najdi ewes. *International Journal of Animal and Veterinary Advances* **2014**, *6*, 28-33.
- 413 7. Caja, G.; Bocquier, F. Effects of nutrition on the composition of sheep's milk. *Cah. Options Mediterr.* **2000**, *55*,  
414 59-74.



- 415 8. Hristov, A.N.; Price, W.J.; Shafii, B. A meta-analysis examining the relationship among dietary factors, dry  
416 matter intake, and milk and milk protein yield in dairy cows. *J. Dairy Sci.* **2004**, *87*, 2184-2196.
- 417 9. Kennelly, J.J.; Bell, J.A.; Keating, A.F.; Doepel, L. *Nutrition as a tool to alter milk composition*; 2005; Vol. 17, pp.  
418 255-275.
- 419 10. Chilliard, Y.; Ferlay, A.; Rouel, J.; Lamberet, G. A review of nutritional and physiological factors affecting  
420 goat milk lipid synthesis and lipolysis. *Livest. Prod. Sci.* **2003**, *86*, 1751-1770.
- 421 11. Pulina, G.; Nudda, A.; Battaccone, G.; Cannas, A. Effects of nutrition on the contents of fat, protein, somatic  
422 cells, aromatic compounds, and undesirable substances in sheep milk. *Anim. Feed Sci. Technol.* **2006**, *131*,  
423 255-291.
- 424 12. McGuire, M.A.; McGuire, M.K. Conjugated linoleic acid (CLA): A ruminant fatty acid with beneficial effects  
425 on human health. *J. Anim. Sci.* **2000**, *77*, 1.
- 426 13. Calder, P.C. Long-chain fatty acids and inflammation. *Proc. Nutr. Soc.* **2012**, *71*, 284-289.
- 427 14. Calder, P.C. Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology?  
428 *Brit. J. Clin. Pharmacol.* **2013**, *75*, 645-662.
- 429 15. Belury, M.A. Inhibition of carcinogenesis by conjugated linoleic acid: potential mechanisms of action. *J.*  
430 *Nutr.* **2002**, *132*, 2995-2998.
- 431 16. Calon, F.; Cole, G. Neuroprotective action of omega-3 polyunsaturated fatty acids against  
432 neurodegenerative diseases: Evidence from animal studies. *Prostaglandins Leukotrienes Essential Fatty Acids*  
433 **2007**, *77*, 287-293.
- 434 17. Bernal-Santos, G.; O'Donnell, A.M.; Vicini, J.L.; Hartnell, G.F.; Bauman, D.E. Hot topic: Enhancing omega-  
435 3 fatty acids in milk fat of dairy cows by using stearidonic acid-enriched soybean oil from genetically  
436 modified soybeans. *J. Dairy Sci.* **2010**, *93*, 32-37.
- 437 18. Castro, T.; Manso, T.; Jimeno, V.; Del Alamo, M.; Mantecon, A.R. Effects of dietary sources of vegetable fats  
438 on performance of dairy ewes and conjugated linoleic acid (CLA) in milk. *Small Ruminant Res.* **2009**, *84*, 47-  
439 53.
- 440 19. Otto, J.R.; Nish, P.; Balogun, R.O.; Freeman, M.J.; Malau-Aduli, B.S.; Lane, P.A.; Malau-Aduli, A.E.O. Effect  
441 of dietary supplementation of pasture-based primiparous Holstein-Friesian cows with degummed crude  
442 canola oil on body condition score, liveweight, milk yield and composition. *Journal of Applied Animal*  
443 *Research* **2016**, *44*, 194-200.
- 444 20. Pirondini, M.; Colombini, S.; Mele, M.; Malagutti, L.; Rapetti, L.; Galassi, G.; Croveto, G.M. Effect of dietary  
445 starch concentration and fish oil supplementation on milk yield and composition, diet digestibility, and  
446 methane emissions in lactating dairy cows. *J. Dairy Sci.* **2015**, *98*, 357-372.
- 447 21. Pulina, G.; Macciotta, N.; Nudda, A. Milk composition and feeding in the Italian dairy sheep. *Italian Journal*  
448 *of Animal Science* **2005**, *4*, 5-14.
- 449 22. Akbaridoust, G.; Plozza, T.; Trenerry, V.C.; Wales, W.J.; Auldist, M.J.; Dunshea, F.R.; Ajlouni, S. Influence  
450 of different systems for feeding supplements to grazing dairy cows on milk fatty acid composition. *J. Dairy*  
451 *Res.* **2014**, *81*, 156-163.
- 452 23. Bell, J.A.; Griinari, J.M.; Kennelly, J.J. Effect of safflower oil, flaxseed oil, monensin, and vitamin E on  
453 concentration of conjugated linoleic acid in bovine milk fat. *J. Dairy Sci.* **2006**, *89*, 733-748.
- 454 24. Lunsin, R.; Wanapat, M.; Rowlinson, P. Effect of cassava hay and rice bran oil supplementation on rumen  
455 fermentation, milk yield and milk composition in lactating dairy cows. *Asian-Australas J Anim Sci* **2012**, *25*,  
456 1364-1373.

- 457 25. Park, J.K.; Kwon, E.G.; Kim, C.H. Effects of increasing supplementation levels of rice bran on milk  
458 production and fatty acid composition of milk in Saanen dairy goats. *Anim. Prod. Sci.* **2013**, *53*, 413-418.
- 459 26. Caroprese, M.; Albenzio, M.; Bruno, A.; Fedele, V.; Santillo, A.; Sevi, A. Effect of solar radiation and flaxseed  
460 supplementation on milk production and fatty acid profile of lactating ewes under high ambient  
461 temperature. *J. Dairy Sci.* **2011**, *94*, 3856-3867.
- 462 27. Caroprese, M.; Ciliberti, M.G.; Marino, R.; Santillo, A.; Sevi, A.; Albenzio, M. Polyunsaturated fatty acid  
463 supplementation: effects of seaweed *ascophyllum nodosum* and flaxseed on milk production and fatty acid  
464 profile of lactating ewes during summer. *J. Dairy Res.* **2016**, *83*, 289-297.
- 465 28. Mughetti, L.; Sinesio, F.; Acuti, G.; Antonini, C.; Moneta, E.; Peparario, M.; Tralbalza-Marinucci, M.  
466 Integration of extruded linseed into dairy sheep diets: Effects on milk composition and quality and  
467 sensorial properties of Pecorino cheese. *Anim. Feed Sci. Technol.* **2012**, *178*, 27-39.
- 468 29. Kenyon, P.R.; Maloney, S.K.; Blache, D. Review of sheep body condition score in relation to production  
469 characteristics. *N. Z. J. Agric. Res.* **2014**, *57*, 38-64.
- 470 30. Mavrogenis, A.P.; Papachristoforou, C. Estimation of the energy value of milk and prediction of fat-  
471 corrected milk yield in sheep. *Small Ruminant Res.* **1988**, *1*, 229-236.
- 472 31. SAS (2009). Statistical Analysis System. *SAS Institute, version 9.2. Cary, NC, USA.*
- 473 32. Hervas, G.; Luna, P.; Mantecon, A.R.; Castanares, N.; de la Fuente, M.A.; Juarez, M.; Frutos, P. Effect of diet  
474 supplementation with sunflower oil on milk production, fatty acid profile and ruminal fermentation in  
475 lactating dairy ewes. *J. Dairy Res.* **2008**, *75*, 399-405.
- 476 33. Ammah, A.A.; Benchaar, C.; Bissonnette, N.; Gevry, N.; Ibeagha-Awemu, E.M. Treatment and post-  
477 treatment effects of dietary supplementation with safflower oil and linseed oil on milk components and  
478 blood metabolites of Canadian Holstein cows. *Journal of Applied Animal Research* **2018**, *46*, 898-906.
- 479 34. Mapato, C.; Wanapat, M.; Cherdthong, A. Effects of urea treatment of straw and dietary level of vegetable  
480 oil on lactating dairy cows. *Trop. Anim. Health Prod.* **2010**, *42*, 1635-1642.
- 481 35. Shingfield, K.J.; Reynolds, C.K.; Hervas, G.; Griinari, J.M.; Grandison, A.S.; Beever, D.E. Examination of the  
482 persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows.  
483 *J. Dairy Sci.* **2006**, *89*, 714-732.
- 484 36. Illius, A.W.; Jessop, N.S. Metabolic constraints on voluntary intake in ruminants. *J. Anim. Sci.* **1996**, *74*, 3052-  
485 3062.
- 486 37. Petit, H.V.; Ivan, M.; Mir, P.S. Effects of flaxseed on protein requirements and N excretion of dairy cows  
487 fed diets with two protein concentrations. *J. Dairy Sci.* **2005**, *88*, 1755-1764.
- 488 38. Gonthier, C.; Mustafa, A.F.; Berthiaume, R.; Petit, H.V.; Martineau, R.; Ouellet, D.R. Effects of feeding  
489 micronized and extruded flaxseed on ruminal fermentation and nutrient utilization by dairy cows. *J. Dairy  
490 Sci.* **2004**, *87*, 1854-1863.
- 491 39. Doreau, M.; Chilliard, Y. Digestion and metabolism of dietary fat in farm animals. *Brit. J. Nutr.* **1997**, *78*,  
492 S15-S35.
- 493 40. Malau-Aduli, A.E.O.; Anlade, Y.R. Comparative study of milk compositions of cattle, sheep and goats in  
494 Nigeria. *Animal Science Journal* **2002**, *73*, 541-544.
- 495 41. Morgan-Davies, C.; Waterhouse, A.; Pollock, M.L.; Milner, J.M. Body condition score as an indicator of ewe  
496 survival under extensive conditions. *Anim. Welfare* **2008**, *17*, 71-77.
- 497 42. Phythian, C.J.; Michalopoulou, E.; Jones, P.H.; Winter, A.C.; Clarkson, M.J.; Stubbings, L.A.; Grove-White,  
498 D.; Cripps, P.J.; Duncan, J.S. Validating indicators of sheep welfare through a consensus of expert opinion.  
499 *Animal* **2011**, *5*, 943-952.



- 500 43. Roche, J.R.; Friggens, N.C.; Kay, J.K.; Fisher, M.W.; Stafford, K.J.; Berry, D.P. Invited review: Body condition  
501 score and its association with dairy cow productivity, health, and welfare. *J. Dairy Sci.* **2009**, *92*, 5769-5801.
- 502 44. Abdel-Mageed, I. Body condition scoring of local Ossimi ewes at mating and its impact on fertility and  
503 prolificacy *Egyptian Journal of Sheep and Goat Sciences* **2009** *4*, 37-44.
- 504 45. Kenyon, P.R.; Morel, P.C.H.; Morris, S.T. The effect of individual liveweight and condition scores of ewes  
505 at mating on reproductive and scanning performance. *New Zealand Veterinary Journal* **2004**, *52*, 230-235.
- 506 46. Yilmaz, M.; Altin, T.; Karaca, O.; Cemal, I.; Bardakcioglu, H.E.; Yilmaz, O.; Taskin, T. Effect of body  
507 condition score at mating on the reproductive performance of Kivircik sheep under an extensive  
508 production system. *Trop. Anim. Health Prod.* **2011**, *43*, 1555-1560.
- 509 47. Komaragiri, M.V.S.; Casper, D.P.; Erdman, R.A. Factors affecting body tissue mobilization in early lactation  
510 dairy cows. 2. Effect of dietary fat on mobilization of body fat and protein. *J. Dairy Sci.* **1998**, *81*, 169-175.
- 511 48. Ricegrowers' Association of Australia (RAG) (2013). *Overview of the Australian rice industry*. Available at:  
512 <http://www.rga.org.au/f.ashx/overview.pdf>
- 513 49. Seymour, M.; Kirkegaard, J.A.; Peoples, M.B.; White, P.F.; French, R.J. Break-crop benefits to wheat in  
514 Western Australia - insights from over three decades of research. *Crop & Pasture Science* **2012**, *63*, 1-16.
- 515 50. Nudda, A.; Battacone, G.; Neto, O.B.; Cannas, A.; Francesconi, A.H.D.; Atzori, A.S.; Pulina, G. Feeding  
516 strategies to design the fatty acid profile of sheep milk and cheese. *Revista Brasileira De Zootecnia-Brazilian*  
517 *Journal of Animal Science* **2014**, *43*, 445-456.
- 518 51. Gomez-Cortes, P.; Gallardo, B.; Mantecon, A.R.; Juarez, M.; de la Fuente, M.A.; Manso, T. Effects of different  
519 sources of fat (calcium soap of palm oil vs. extruded linseed) in lactating ewes' diet on the fatty acid profile  
520 of their suckling lambs. *Meat Sci.* **2014**, *96*, 1304-1312.
- 521 52. Nudda, A.; Correddu, F.; Marzano, A.; Battacone, G.; Nicolussi, P.; Bonelli, P.; Pulina, G. Effects of diets  
522 containing grape seed, linseed, or both on milk production traits, liver and kidney activities, and immunity  
523 of lactating dairy ewes. *J. Dairy Sci.* **2015**, *98*, 1157-1166.
- 524 53. Brossillon, V.; Reis, S.F.; Moura, D.C.; Galvao, J.G.B.; Oliveira, A.S.; Cortes, C.; Brito, A.F. Production, milk  
525 and plasma fatty acid profile, and nutrient utilization in Jersey cows fed flaxseed oil and corn grain with  
526 different particle size. *J. Dairy Sci.* **2018**, *101*, 2127-2143.
- 527 54. Li, R.; Beaudoin, F.; Ammah, A.A.; Bissonnette, N.; Benchaar, C.; Zhao, X.; Lei, C.Z.; Ibeagha-Awemu, E.M.  
528 Deep sequencing shows microRNA involvement in bovine mammary gland adaptation to diets  
529 supplemented with linseed oil or safflower oil. *BMC Genomics* **2015**, *16*.
- 530 55. Nudda, A.; Battacone, G.; Atzori, A.S.; Dimauro, C.; Rassa, S.P.G.; Nicolussi, P.; Bonelli, P.; Pulina, G. Effect  
531 of extruded linseed supplementation on blood metabolic profile and milk performance of Saanen goats.  
532 *Animal* **2013**, *7*, 1464-1471.
- 533 56. Glibert, J.S.; Porter, T. International safflower production-an overview. In Proceedings of Safflower:  
534 unexploited potential and world adaptability. 7th International Safflower Conference, Wagga Wagga, New  
535 South Wales, Australia; pp. 1-7.
- 536 57. Alizadeh, A.R.; Alikhani, M.; Ghorbani, G.R.; Rahmani, H.R.; Rashidi, L.; Loor, J.J. Effects of feeding roasted  
537 safflower seeds (variety IL-111) and fish oil on dry matter intake, performance and milk fatty acid profiles  
538 in dairy cattle. *J. Anim. Physiol. Anim. Nutr.* **2012**, *96*, 466-473.
- 539 58. Shingfield, K.J.; Bonnet, M.; Scollan, N.D. Recent developments in altering the fatty acid composition of  
540 ruminant-derived foods. *Animal* **2013**, *7*, 132-162.

- 541 59. Ahmadpour, A.; Aliarabi, H.; Khan, M.G.; Patton, R.A.; Bruckmaier, R.M. Temporal changes in milk fatty  
542 acid distribution due to feeding different levels of rolled safflower seeds to lactating Holstein cows. *J. Dairy*  
543 *Sci.* **2017**, *100*, 4484-4499.
- 544 60. Dschaak, C.M.; Noviandi, C.T.; Eun, J.S.; Fellner, V.; Young, A.J.; ZoBell, D.R.; Israelsen, C.E. Ruminant  
545 fermentation, milk fatty acid profiles, and productive performance of Holstein dairy cows fed 2 different  
546 safflower seeds. *J. Dairy Sci.* **2011**, *94*, 5138-5150.
- 547 61. Oguz, M.N.; Oguz, F.K.; Buyukoglu, T.I. Effect of different concentrations of dietary safflower seed on milk  
548 yield and some rumen and blood parameters at the end stage of lactation in dairy cows. *Revista Brasileira*  
549 *De Zootecnia-Brazilian Journal of Animal Science* **2014**, *43*, 207-211.
- 550 62. Shi, H.P.; Luo, J.; Zhang, W.; Sheng, H.J. Using safflower supplementation to improve the fatty acid profile  
551 in milk of dairy goat. *Small Ruminant Res.* **2015**, *127*, 68-73.
- 552 63. Balthazar, C.F.; Pimentel, T.C.; Ferrão, L.L.; Almada, C.N.; Santillo, A.; Albenzio, M.; Mollakhalili, N.;  
553 Mortazavian, A.M.; Nascimento, J.S.; Silva, M.C., et al. Sheep Milk: Physicochemical Characteristics and  
554 Relevance for Functional Food Development. *Comprehensive Reviews in Food Science and Food Safety* **2017**, *16*,  
555 247-262.
- 556 64. Naik, P.K. Bypass Fat in Dairy Ration - A Review. *Animal Nutrition and Feed Technology* **2013**, *13*, 147-163.
- 557 65. Appeddu, L.A.; Ely, D.G.; Aaron, D.K.; Deweese, W.P.; Fink, E. Effects of supplementing with calcium salts  
558 of palm oil fatty acids or hydrogenated tallow on ewe milk production and twin lamb growth. *J. Anim. Sci.*  
559 **2004**, *82*, 2780-2789.
- 560 66. Garcia, C.D.; Hernandez, M.P.; Cantalapiedra, G.; Salas, J.M.; Merino, J.A. Bypassing the rumen in dairy  
561 ewes: The reticular groove reflex vs. calcium soap of olive fatty acids. *J. Dairy Sci.* **2005**, *88*, 741-747.
- 562 67. Kitessa, S.M.; Peake, D.; Bencini, R.; Williams, A.J. Fish oil metabolism in ruminants. *Anim. Feed Sci. Technol.*  
563 **2003**, *108*, 1-14.
- 564 68. Rotunno, T.; Sevi, A.; Di Caterina, R.; Muscio, A. Effects of graded levels of dietary rumen-protected fat on  
565 milk characteristics of Comisana ewes. *Small Ruminant Res.* **1998**, *30*, 137-145.
- 566 69. Haenlein, G.F.W. About the evolution of goat and sheep milk production. *Small Ruminant Res.* **2007**, *68*, 3-  
567 6.
- 568 70. Gootwine, E.; Goot, H. Lamb and milk production of Awassi and East-Friesian sheep and their crosses  
569 under Mediterranean environment. *Small Ruminant Res.* **1996**, *20*, 255-260.
- 570 71. Konečná, L.; Kuchtík, J.; Králíčková, Š.; Pokorná, M.; Šustová, K.; Filipčík, R.; Lužová, T. Effect of different  
571 crossbreeds of Lacaune and East Friesian breeds on milk yield and basic milk parameters. *Acta Universitatis*  
572 *Agriculturae et Silviculturae Mendelianae Brunensis* **2013**, *61*, 93-98.
- 573 72. Thomas, D.L.; Berger, Y.M.; McKusick, B.C. Milk and lamb production of East Friesian-cross ewes in  
574 northwestern Wisconsin. In Proceedings of Proc. 4th Great Lakes Dairy Sheep Symp; pp. 11-17.
- 575 73. Galal, S.; Gursoy, O.; Shaat, I. Awassi sheep as a genetic resource and efforts for their genetic improvement-  
576 A review. *Small Ruminant Res.* **2008**, *79*, 99-108.
- 577 74. Stubbs, A.; Abud, G.; Bencini, R. Dairy sheep Manual: Farm management guidelines. Rural Industries  
578 research and Development Corporation: 2009.
- 579 75. Clement, P.; Agboola, S.O.; Bencini, R. A study of polymorphism in milk proteins from local and imported  
580 dairy sheep in Australia by capillary electrophoresis. *Lwt-Food Science and Technology* **2006**, *39*, 63-69.
- 581 76. Silanikove, N. Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livest. Prod.*  
582 *Sci.* **2000**, *67*, 1-18.
- 583 77. West, J.W. Effects of heat-stress on production in dairy cattle. *J. Dairy Sci.* **2003**, *86*, 2131-2144.