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RESEARCH NOTE

Feeding extruded linseed to dairy ewes under extensive grazing conditions

Einar Vargas-Bello-Pérez, Raúl R. Vera, Claudio Aguilar, Rodrigo Lira,
Iván Peña, and Franco. A. Tello

Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile. Casilla-306,
Santiago, Chile.

Abstract

E. Vargas-Bello-Pérez, R. R. Vera, C. Aguilar, R. Lira, I. Peña, and F.A. Tello. 2014. Feeding extruded linseed to dairy ewes under extensive grazing conditions. *Cien. Inv. Agr. 41(1): 115-122.* The aim of this trial was to evaluate the effect of dietary supplementation of extruded linseed on the milk and cheese fatty acid (FA) profiles of ewes under extensive grazing conditions. Over 26 days, a group of nine lactating ewes (Latxa × Milchschaaf × Corriedale) in mid-lactation were managed under grazing conditions and given supplements during each manual milking (0830 and 1800 h) of 50% corn and 50% oats (500 g ewe⁻¹ milking⁻¹; control, TC) for the first 6 days. From day 7 to 20, the supplement was 25% corn, 25% oats and 50% extruded linseed (500 g ewe⁻¹ milking⁻¹) (extruded linseed; TEL). From day 21 to 26, the ewes were fed TC. The saturated FA content in milk was reduced (3.6%), and the monounsaturated, polyunsaturated and n-3 FA (7, 10 and 25%, respectively) were increased when the ewes were supplemented with extruded linseed. A correlation between rumenic acid with vaccenic acid ($R^2=0.68$) was found in the milk fat from ewes fed TEL. In cheese obtained from TEL, the contents of n-3 and polyunsaturated FA were increased (21 and 7%, respectively). In conclusion, a supplement of extruded linseed in ewes under grazing conditions could be an alternative lipid source to produce cheese with additional nutritional value. However, the effect of extruded linseed supplementation in ewes under the same conditions with longer experimental periods and a more suitable statistical design must be investigated.

Key words: Extruded linseed, n-3 fatty acids, cheese, ewe, milk.

Introduction

In recent decades, the potential positive health effects (anti-carcinogenic, anti-atherogenic and anti-obesity) of certain milk fatty acids (FA) such as n-3 FA and conjugated linoleic acid (CLA; C18:2 c9, t11) that are observed in dairy products have stimulated an interest in improving milk FA (Vargas-Bello-Pérez

and Garnsworthy, 2013). Because oilseeds are rich in C18:3n-3 and C18:2n-6, their inclusion in ruminant diets can increase the concentrations of specific health-promoting FA such as C18:3n-3 and C18:2 c9, t11 in milk (Manso *et al.*, 2011). Ewe milk fat is an important natural source of CLA (approximately 1% of total FA; Luna *et al.*, 2005), and rumenic acid (RA; C18:2 c9, t11) is the main CLA isomer, and it comprises almost 90% of the total CLA in ruminant milk fat (Zhang *et al.*, 2006).

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Corresponding author: evargasb@uc.cl

Different studies have reported the effects of whole linseed (Luna *et al.*, 2005), linseed oil (Bouattour *et al.*, 2006) and extruded linseed (Gomez-Cortes *et al.*, 2009; Mele *et al.*, 2007, 2011; Mughetti *et al.*, 2012; Branciari *et al.*, 2012) on milk FA composition in ewes. However, few studies have investigated ewes that are supplemented with extruded linseed under extensive grazing conditions. The present trial was conducted to enhance ewes' milk fat by dietary means (extruded linseed) in a short time period. Furthermore, research on this topic could lead to the development of natural, consumer-friendly strategies and processing systems to produce CLA/n-3 enriched dairy foods with enhanced nutritional properties. Therefore, a 26-d trial was conducted to evaluate the effect of dietary supplementation of extruded linseed on the milk and cheese FA profile of ewes under extensive grazing conditions.

Materials and methods

Animals and treatments

The trial was performed from December 2010 to January 2011 in Ancud, Chile (42°1'6" S 73°50'7" W). Over 26 days, a group of nine lactating multiparous ewes ($n=9$) (50% Latxa $\times$ 25% Milchschaef $\times$ 25% Corriedale) with 90 d of lactation at the onset of the trial were managed under grazing conditions and supplemented during each manual milking (0830 and 1800 h) with 50% corn and 50% oats (500 g⁻¹ milking⁻¹) (the control was the usual supplement, denoted TC) during the first 6 days; from day 7 to 20, the supplement was based on 25% corn, 25% oats and 50% extruded linseed (500 g ewe⁻¹ milking⁻¹) (extruded linseed; TEL), and from day 21 to 26, the ewes were fed TC. There was no adaptation period, and ewes were separated from their lambs before the trial. Ewes were allocated to a grazing paddock (7000 m²) with free access to water.

Measurements, sample collection and chemical analyses

Milk samples (300 mL) from each of the nine ewes were collected daily from the first milking (0830 h) in aliquots of 100 mL (preserved with 100 mg of K₂Cr₂O₇) and stored at -20 °C for later analysis. The supplements were sampled every two days and stored at -20 °C for later chemical analysis. The standard procedures described by the Association of Official Analytical Chemists (AOAC, 2006) were used to determine the Kjeldahl N (984.13) and ether extract (920.39). The crude protein was calculated as N $\times$ 6.25, and the neutral detergent fiber was determined according to the methods described by Van Soest *et al.* (1991). Milk samples were analyzed for pH, titratable acidity (Thörner degrees; Th), fat content (Gerber method; British Standards Institution 696, 1969), crude protein (16.036; Kjeldahl N $\times$ 6.38), total ash (16.035) and solids (16.032) according to the AOAC (1984) procedures.

Grassland species characterization

At the beginning of the trial, a line-intercept method (Sutherland, 2006) with transect lines was used to measure the cover and characterize the grassland species. Cover was estimated by measuring the length of the transect line occupied by each species and expressing it as a percentage of the total length. Each transect was 2-m length with 10-cm interval points, and they were distributed in random locations within the experimental paddock.

Cheese-making process

Cheese was produced from 4 consecutive milking times (2 from 0800 h and 2 from 1800 h) over 2 days in which 10 L of raw milk was heated to 32 °C (at a rate of 1 °C 3 min⁻¹), and a commercial rennet was added to curdle the milk at 38 °C. No

starter culture was added to produce the cheese because raw milk was used. After the milk had clotted (40 min), the curd was cut to the size of a hazelnut and the vat temperature was gradually increased to 37 °C at a rate of 1 °C 3 min⁻¹. At the same time, the curd was stirred to remove the whey and favor grain aggregation. Curds were placed into molds (16 cm diameter and 10 cm height) and pressed in a horizontal mechanic press until the pH was approximately 7.2. The cheese was salted in brine at 12 °C for 15 h and then transferred to a ripening room where it remained at a temperature of 12 °C and ~85% RH for 120 d; afterwards, two cores of each cheese were obtained for FA analysis.

Fatty acid analysis

The analyses of the fatty acid methyl esters (FAME) from the experimental diets, extruded linseed, milk and cheese samples were performed using a gas chromatograph (GC; Shimadzu Scientific Instruments AOC-20s, Columbia, MD, USA) equipped with a 100 m × 0.32 mm × 0.20 µm column (Rtx-2560). The lipid extraction / methylation and the GC conditions were similar to those reported by Vargas-Bello-Pérez *et al.* (2013). The fatty acid peaks were identified by using the Food Industry 37 FAME Mix (Restek Co, Bellefonte, PA, USA).

The nature of this trial and limited availability of on-farm facilities led to a “before and after” sequence of treatments applied to the whole group of experimental animals. A quasi-experimental design was used that included baseline data (first 6 and last 6 days of the trial) to measure the outcome before the intervention (lipid supplementation) and follow-up data to determine the outcome after a passage of time that was deemed sufficient (14 d) for the impact of the intervention to set in. The animal performance traits and chemical parameters of the milk and cheese were subjected to a one-way ANOVA analysis to compare the effects of the control and experimental diets using the

SAS Statistical Package (2004, SAS Inst. Inc., Cary, NC.). A probability of $P < 0.05$ was used to determine significant differences among means.

Results and discussion

The chemical composition of the dietary treatments is reported in Table 1. As expected, the most predominant FAs found in the extruded linseed were C18:2n-6 (17.3 g 100 g⁻¹) and C18:3n-3 (51.9 g 100 g⁻¹). The main species found in the experimental paddock (which represented 85% of the total grassland species coverage) were *Agrostis tenuis*, *Trifolium repens* and *Anthoxanthum odoratum*, whereas *Lotus uliginosus* contributed only 2%. The remaining grassland species found in the paddock were identified as *Dichondra repens*, *Juncus bufonius*, *Taraxacum officinale* and *Ranunculus repens*.

Table 1. Chemical composition of dietary treatments (DM basis).

Chemical composition (g kg ⁻¹)	Control ¹	Extruded linseed ²	Extruded linseed
Crude fiber	89	122	155
Ether extract	46	116	187
Crude protein	91	179	266
Neutral detergent fiber	236	232	229
Ash	19	31	44

¹50% corn + 50% oats (1000 g ewe⁻¹ d⁻¹); ²25% corn + 25% oats + 50% extruded linseed (1000 g ewe⁻¹ d⁻¹).

The milk yield was not affected by the inclusion of extruded linseed; however, the total solids, milk fat and pH were increased by TEL (Table 2). Luna *et al.* (2005) reported that there was no difference in milk yield and a decreased milk fat content when ewes were fed with a supplement enriched in whole linseed. In addition, no differences in milk fat content were reported by Gomez-Cortés *et al.* (2009) when ewes were fed extruded linseed (12% DM). Usually, the dietary lipids in ruminant rations contributed significantly to the energy density of the diet, which is reflected in an increased milk yield (Akraim *et al.*, 2006). The supplementation of TEL affected some of the physical characteristics of milk, and despite

the increase in milk pH, this value is within the physiological range reported for sheep milk (Park *et al.*, 2007). The titratable acidity, a quality indicator of raw milk, was reduced, which suggested a lower content of molecules with buffering capacity, such as the casein fractions (Bianchi *et al.*, 2004).

Table 2 Milk yield and milk composition of ewes receiving a diet supplemented with extruded linseed.

Item	Control ¹	Extruded linseed ²	P-value ³
Milk yield, kg d ^{-1.4}	12.07±0.41	12.19±0.30	0.051
Milk composition, %			
Total solids	18.54 ^b ±0.16	18.95 ^a ±0.08	0.028
Protein	4.02±0.10	3.98±0.02	0.249
Fat	6.82 ^b ±0.33	7.18 ^a ±0.07	0.009
Ash	1.01±0.01	1.00±0.01	0.539
Titratable acidity, °Th	13.40 ^a ±0.33	12.00 ^b ±0.20	0.001
pH	6.47 ^b ±0.02	6.56 ^a ±0.03	0.017

¹50% corn + 50% oats (1000 g ewe⁻¹ d⁻¹); ²25% corn + 25% oats + 50% extruded linseed (1000 g ewe⁻¹ d⁻¹). ³P-value represents the probability of a treatment effect. ⁴Milk yield from 2 milking times from 9 ewes. ^{a, b}Means in the same row with different superscripts are different (P≤0.05).

Milk and cheese fatty acid composition

The inclusion of TEL reduced the milk C16:0 and increased the C18:1 t11. Compared to TC, TEL increased the C18:2 t9, t11 and C18:3n-3 (Table 3). In this trial, increases in C18:2 isomers and C18:1 t11 contents in milk are most likely a result of the extensive hydrolysis and biohydrogenation occurring in the rumen. Two of the biohydrogenation intermediates that escape from the rumen are C18:1 t11 (vaccenic acid; VA), which is formed from both C18:2n-6 and C18:3n-3; and RA, which is formed during the biohydrogenation of linoleic acid (Vargas-Bello-Pérez and Garnsworthy, 2013) that is supplied by extruded linseed.

In this trial, the inclusion of extruded linseed reduced the milk saturated fatty acids (SFA) by 3.6%. Mele *et al.* (2007) reported a 9.6% decreased in milk SFA content when grazing ewes were supplemented with 30% DM extruded linseed. Additionally, Gomez-Cortés *et al.* (2009) reported an 18% reduction of milk SFA when ewes were fed

with extruded linseed. The fatty acids from C6:0 to C14:0 are synthesized *de novo* in the mammary gland, whereas C16:0 is derived from both the diet and *de novo* synthesis. A substantial part of the reduction in *de novo* synthesis of FA as a result of feeding PUFA (*e.g.*, extruded linseed) could be as a result of an increased uptake of dietary and ruminally derived FA (Vargas-Bello-Pérez and Garnsworthy, 2013).

The inclusion of TEL increased the amount of C18:2 t9, t11 and C18:3n-3 in the produced cheese (Table 3). The content of C18:3n-3, an intermediate product of the rumen biohydrogenation, was higher in TEL milk and cheese, which suggested that feeding extruded linseed to dairy ewes resulted in the ruminal accumulation of this FA, which may partly escape from the rumen and be transferred to milk and cheese (Plourde *et al.*, 2007). Mele *et al.* (2011) also reported increases in cheese C18:3n-3 when ewes were fed a concentrate with 30% extruded linseed.

The saturated FA and thrombogenic index were decreased, and the monounsaturated FA, polyunsaturated FA, and polyunsaturated n-3 and n-6 FA were increased in milk. However, only the polyunsaturated FA and polyunsaturated n-3 and n-6 FA were increased in cheese of ewes supplemented with TEL (Table 3). Although, the atherogenicity and thrombogenic indexes of the cheese were not affected by TEL, the atherogenicity index was lower (1.9 vs. 2.9) than that reported by Abilleira *et al.* (2009), who evaluated the effect of season of the year on the changes in management system of the commercial sheep flocks in the FA profile of Idiazabal cheese (a type of cheese similar to those elaborated in this trial). Furthermore, the data reported in Table 3 shows that throughout the trial, the atherogenicity index of the control diet tended to increase significantly (P≤0.05), whereas the diet of TEL showed a significant decreasing trend (P≤0.05). It should be noted that the FA composition of the local forage species and the effect of season on their lipid composition have not been determined

Table 3. Fatty acid composition in milk and cheese from ewes receiving a diet supplemented with extruded linseed.

Fatty acid (g 100 g ⁻¹)	Control ¹	Extruded linseed ²	P-value ³
Milk			
C12:0	3.77±0.11	3.54±0.12	0.188
C14:0	11.52±0.32	11.16±0.22	0.357
C16:0	26.16 a±0.55	24.69 b±0.19	0.021
C18:0	13.83±0.61	14.58±0.31	0.277
C18:1 t11	5.41 b±0.11	6.00 a±0.11	0.004
C18:1 c9	18.58±0.50	18.28±0.39	0.638
C18:2 c9, t11	2.35±0.08	2.44±0.08	0.476
C18:2n-6	1.43±0.02	1.45±0.03	0.552
C18:3n-3	0.95 b±0.06	1.19 a±0.05	0.007
Saturated fatty acids	68.55 a±0.72	66.05 b±0.60	0.021
Monounsaturated (MUFA)	26.95 b±0.66	28.81 a±0.54	0.049
Polyunsaturated (PUFA)	5.24 b±0.09	5.77 a±0.06	<0.001
PUFA n-3/n-6	2.09±0.12	1.82±0.06	0.066
Atherogenicity index ⁴	2.37±0.11	2.11±0.06	0.055
Thrombogenic index ⁵	2.91 a±0.09	2.58 b±0.04	0.003
Cheese			
C10:0	4.86±0.27	4.87±0.16	0.971
C12:0	3.4±0.12	3.22±0.09	0.259
C14:0	10.56±0.43	10.58±0.12	0.969
C16:0	25.17±0.60	24.41±0.16	0.217
C18:0	14.69±0.58	14.89±0.22	0.744
C18:1 t11	5.82±0.16	6.15±0.11	0.111
C18:1 c9	19.52±0.59	19.06±0.28	0.476
C18:2 c9, t11	2.59±0.05	2.59±0.07	0.946
C18:2n-6	1.48±0.01	1.50±0.02	0.626
C18:3n-3	0.96 b±0.05	1.16 a±0.05	0.013
Saturated fatty acids	65.31±0.72	63.99±0.45	0.138
Monounsaturated (MUFA)	29.14±0.66	30.06±0.37	0.233
Polyunsaturated (PUFA)	5.58 b±0.09	5.96 a±0.11	0.027
PUFA n-3/n-6	2.14±0.25	1.91±0.14	0.069
Atherogenicity index ⁴	2.03±0.26	1.94±0.09	0.426
Thrombogenic index ⁵	2.66±0.21	2.48±0.09	0.060

¹50% corn + 50% oats (1000 g ewe⁻¹ d⁻¹); ²25% corn + 25% oats + 50% extruded linseed (1000 g ewe⁻¹ d⁻¹). ³P-value represents the probability of a treatment effect. ⁴Atherogenicity index = [(12:0 + 4(14:0) + 16:0) / [(n6+n3)PUFA + 18:1 + ΣMUFA] (Ulbricht and Southgate, 1991); ⁵Thrombogenic index = (14:0+16:0+18:0) / [(0.5x18:1) + 0.5(ΣMUFA) + 0.5(n6PUFA) + 3(n3PUFA) + (n3PUFA/n6PUFA)] (Ulbricht and Southgate, 1991). ^{a, b}Means in the same row with different superscripts are different (P≤0.05).

to date. Plant species and season have been shown to markedly influence the FA composition, and these variations are reflected in the sheep milk fat composition (Mel'uchova *et al.*, 2008).

Ruminic acid (C18:2 c9, t11) and vaccenic acid (C18:1 t11) relationship

Throughout the 26-d trial, the milk FA profile was altered by TEL. Figure 1 shows the temporal patterns that occurred in C18:0, C18:1 t11, C18:2 c9, t11 and C18:3n-3 when ewes were fed extruded linseed. In this trial, the temporal patterns of milk were similar to those reported by Luna *et al.* (2005) in which an increase in C18:2 c9, t11 in milk of ewes supplemented with whole linseed (17% DM) was observed; however, they reported an increase in C18:0 and C18:3n-3, whereas in this trial, the concentrations of C18:0 remained constant with a slight tendency to be reduced and C18:3n-3 was increased within the first days post supplementation with TEL and then reduced after the 10th day.

The ruminic acid content in milk fat was not affected by TEL. In this trial, a significant correlation of ruminic acid with vaccenic acid ($Y = 0.6002x - 1.089$, $R^2 = 0.6854$) was found in milk fat from ewes fed TEL. This correlation value was lower than the value reported by Nudda *et al.* (2005), Luna *et al.* (2005, 2008) and Gomez-Cortes *et al.* (2009) ($R^2=0.97, 0.94, 0.98$ and 0.87 , respectively). There are several factors that may intervene in these differences, such as the number of animals used in this trial versus the larger flocks used by Luna *et al.* (2005, 2008) and Nudda *et al.* (2005).

High levels of dietary lipids often induce time-dependent ruminal adaptations that lead to alterations in the formation of specific biohydrogenation intermediates (e.g., C18:2 c9, t11) (Vargas-Bello-Pérez and Garnsworthy, 2013). In our trial, ewes were supplemented extruded linseed for 14 days, whereas the other studies used longer periods of time (Gomez-Cortes

Figure 1. Temporal pattern (g 100 g⁻¹) of C18:0, C18:1 t11, C18:2 c9, t11 and C18:3n-3 of milk fat from ewes receiving a diet supplemented (days 7 to 20; broken lines) with extruded linseed.

et al., 2009; 60 days and Mele *et al.*, 2011; 10 weeks). Mele *et al.* (2007) have shown that when feeding 30% DM extruded linseed, the CLA contents reached their highest concentration after 4 weeks of supplementation, whereas n-3 FA reached its maximum content after 7 weeks of feeding. Therefore, the long-term lipid supplementation of extruded linseed is required to test the persistency of the level of obtained RA. It is known that dairy ewes grazing on pasture in the vegetative phase (Nudda *et al.*, 2005) can enhance the RA content in ewe's milk; however, the feeding strategy used in this trial could be

adopted when pasture is not readily available for dairy ewes to achieve a constant level of RA enrichment in milk fat.

periods and a more suitable statistical design must be investigated.

Overall, the supplementation (500 g ewe⁻¹ milking⁻¹) of extruded linseed in ewes under grazing conditions did not have detrimental effects on the milk and cheese FA profile. The effect of extruded linseed supplementation in ewes under the same conditions with longer experimental

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Resumen

E. Vargas-Bello-Pérez, R. R. Vera, C. Aguilar, R. Lira, I. Peña y F. A. Tello. 2014. Ovejas en pastoreo extensivo suplementadas con extruido de linaza. Cien. Inv. Agr. 41(1): 115-122.

El objetivo de este ensayo fue evaluar el efecto de suplementar extruido de linaza en el perfil de ácidos grasos de leche y queso de ovejas en pastoreo extensivo. Durante 26 días, un grupo de nueve ovejas lactantes (Latxa × Milchschaef × Corriedale) fueron manejadas en pastoreo extensivo y suplementadas durante cada ordeño manual (830 y 1800 h) con 50% maíz + 50% avena (500 g oveja⁻¹ ordeño⁻¹) durante los primeros 6 días (control; TC), de los días 7 al 20 se suplementaron con 25% maíz + 25% avena + 50% extruido de linaza (500 g oveja⁻¹ ordeño⁻¹) (extruido de linaza; TEL), y finalmente, del día 21 al 26 las ovejas fueron alimentadas con TC. El contenido de ácidos grasos saturados en leche fue reducido (3,6%) mientras que los ácidos grasos monoinsaturados, poliinsaturados y n-3 fueron incrementados (7, 10 y 25%) en aquellas ovejas alimentadas con TEL. Se observó una correlación de ácido ruménico con ácido vaccénico ($R^2=0,68$) en leche de ovejas alimentadas con TEL. Los quesos elaborados de ovejas alimentadas con TEL, aumentaron su contenido de n-3 y ácidos grasos poliinsaturados (21 y 7%). En conclusión, el suplementar ovejas en pastoreo extensivo con extruido de linaza puede ser una alternativa que puede resultar en la elaboración de quesos con un valor nutricional más saludable desde el punto de vista humano. Además resulta necesario investigar más sobre el uso del extruido de linaza bajo las mismas condiciones con periodos más largos de experimentación usando un diseño estadístico más apropiado.

Palabras clave: Ácidos grasos n-3, Extruido de linaza, leche, oveja, queso.

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