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Effects of Ewe's Diet Supplementation with Polyunsaturated Fatty Acids on Meat Lipid Profile of Suckling Lambs

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Abstract: Polyunsaturated fatty acid (PUFA) deposition in lambs' muscles could be influenced by their mothers' diet. The aim was to study the profile of fatty acids in the muscle of lambs from ewes supplemented with different sources of PUFA to achieve a healthier meat for the consumer. On day 100 of gestation, pregnant ewes grazed on natural grass were divided into three groups (n = 20) and supplemented with PUFA: Group A: 700 g of a ration rich in PUFA, Group B: 700 g of a ration for sheep + 20 mL of fish oil and Group C: 700 g of the same ration. After parturition, each group was subdivided: ten ewes continued with the same diet until the end of lactation; the other ten were fed only natural grass. The values of n-3 and n-6 PUFA and alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), linoleic acid (LA) and arachidonic acid (AA) were analyzed in Longissimus lumborum muscles of lambs at 90 and 120 days of life. The feeding of ewes during lactation favorably influenced the lipid profile of the lamb muscle, increasing the concentration of n-3 and n-6 PUFA, ALA, LA, AA, DHA and EPA. The supplementation of ewes with fish oil and/or a ration rich in PUFA improved the LA/ALA ratio in lambs' meat.

Keywords: polyunsaturated fatty acids; n-3 fatty acid; n-6 fatty acid; ewes; lambs; meat; linseed; fish oil

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1. Introduction

The amount of fat, like the composition of fatty deposits, has a significant influence on the quality of the lamb carcass. The fat of the carcass contributes significantly to the technological and sensorial quality of the meat; in addition, it provides energy and essential fatty acids to the human diet and facilitate the absorption of liposoluble vitamins [1].

Positive attributes, sensory and nutritional, of ruminant meat have been overshadowed in recent times by the perception that sheep meat is a high-saturated-fat food with negative effects on consumer health [2]. Fatty acids (FA), present in ruminant meat and considered beneficial to human health, are long-chain polyunsaturated fatty acids (PUFA), in particular those of the n-3 series (DHA, EPA and CLA) [1–3]. The consumption of these long-chain polyunsaturated fatty acids (PUFA) has an important influence on human health, mainly in the prevention of some of the most relevant chronic diseases, such as cardiovascular diseases, neurological diseases (Alzheimer's disease), inflammatory diseases (Crohn's disease), atherosclerosis, diabetes mellitus, arterial hypertension, obesity and infertility, and tumor prevention [2,4–8].

Sheep naturally consume low amounts of PUFA [9]; in addition, the passage of these fatty acids to the duodenum is diminished by the high biohydrogenation that they undergo in the rumen by the action of ruminant bacteria [9–11]. Moreover, in sheep, elongation and desaturation of ALA and LA in tissues are very limited [12]. A possible alternative to modify both the amount and the proportions of FA present in intramuscular fat, seeking to make it healthier for people, is to manipulate the diet consumed by ruminants [1,2,13–16]. Fetal fatty acid profile is directly related to the fatty acid composition of the mother's blood, and therefore to that of the fat that it ingests in the diet. Thus, the feeding of the mother during the final phase of pregnancy with a fish oil supplement (rich in PUFA) determines the composition of the different tissues and organs of the fetus, providing a carcass enriched in n-3 PUFA and CLA [7].

Fats ingested by suckling lambs are not hydrogenated prior to absorption, as occurs in ruminants, so changes in the fatty acid profile of milk produced by ewes are capable of causing changes in characteristics and composition of meat fat, offering the possibility of improving the quality of its carcass [14,15]. Since the composition of the maternal feed, both in type and quantity of fat, allows for the manipulation of the composition of the different tissues and organs of the lamb, the incorporation of fatty supplements into the feed of animals that produce food, and particularly in ruminants, in addition to increasing the energy content of the ration, allows for the manipulation of the fatty acid composition of the lipids of the of lambs, improving the carcass value [2,3,17].

The intramuscular fat of the lambs present a high content of PUFA compared to other fatty deposits; additionally, the levels of eicosapentaenoic acid (EPA) and arachidonic acid (AA) are 25 and 30 times higher, respectively, than in the subcutaneous fat of the suckling lambs. These fatty acids are mainly incorporated into the fraction of phospholipids in the muscle and are found in lesser amounts in adipose tissue triglycerides [1]. Intramuscular fat, located between muscle fibers, is one of the most important for food, since n-3 and n-6 PUFA are located mainly in the phospholipids present in the muscle (between 20 and 50% of the total fatty acids present in phospholipids are long-chain fatty acids of 18, 20 and 22 carbon atoms and 2 to 6 double bonds) [1]. In young animals, the fatty acid composition of the phospholipids has a great influence on the total fatty acid profile of the meat, while as the age of the lamb increases, and therefore the body fat increases, the composition of the triglycerides predominates over the total fatty acid composition [18].

In recent years, attempts have been made to manipulate the levels of different fatty acids in the composition of meat and dairy products of ruminants, with particular emphasis on n-3 PUFA such as ALA, EPA and DHA [11,19]. Therefore, the aim of this research is studying the relationship between Corriedale ewes supplemented with PUFA during the last third of gestation and the initial phase of lactation, correlating these values with the deposition of fatty acids in the muscle of their lambs, measured at 90 and 120 days of age, and trying to modify the values of n-3 fatty acids, in order to achieve a healthier meat for the consumer.

2. Materials and Methods

The experimental trial was carried out at Campo Experimental n° 2 of the Faculty of Veterinary Medicine of the University of Montevideo, located in Libertad, Department of San José (34°38′ S; 56°39′ W), Uruguay, with the approval of the Commission on Ethics in the Use of Animals of the Faculty of Veterinary Medicine (CEUAFVET-1692-111900-000011-23).

2.1. Animals and Experimental Design

Ninety Corriedale adult ewes (n = 90) aged 4–6 years were randomly selected from a flock under the usual production conditions. At the beginning of the experiment, all ewes were in similar physical condition; scores were 2.5 and 3.0 on a 1–5 scale, and the mean body weight was 56 ± 8 kg. Ewes' estrous cycles were synchronized using intravaginal devices with 160 mg of progesterone (Cronipres® CO, Biogénesis-Bagó, Argentina) inserted for 12 days, and then the ewes were left with three fertile rams of the same breed for 4 days

until pregnancy was achieved. The mating date was recorded as day 0 of gestation and was confirmed with transabdominal ultrasonography between days 50 and 70 of gestation. Non-pregnant ewes and those carrying two or more fetuses were removed; afterwards, 60 ewes carrying a single fetus were included in the protocol. The experimental design is shown graphically in Figure 1.

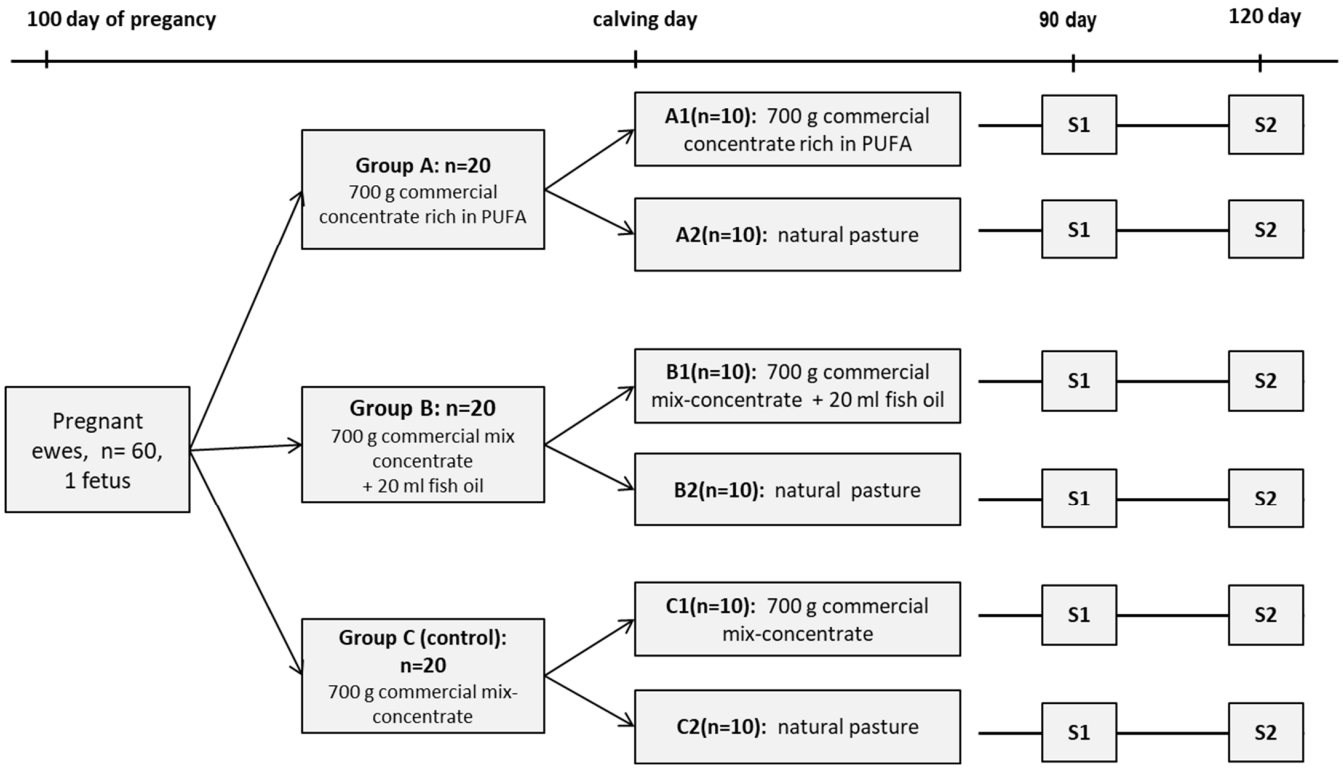

Figure 1. Experimental protocol. n-3 polyunsaturated fatty acids (n-3 PUFA). Group A: fed with 700 g of a diet rich in PUFA before birth; Group B: fed with 700 g of the commercial mix-concentrate together with 20 mL of Vita-Mega3® before birth; Group C: fed with 700 g of the commercial mix-concentrate; Group A1, B1 and C1: fed after birth the same as the prepartum; Group A2, B2 and C2: fed only on natural pasture during the same time period. S-1: sample 1 at 90 days; S-2: sample 2 at 120 days.

Ewes were grazed on meadow pasture composed mainly of Bermuda grass (*Cynodon dactylon*). Every 100 g of grass dry matter provided 9.02% crude protein and 1.78 Mcal metabolizable energy/kg dry matter (Laboratory of Nutrition Faculty of Veterinary Medicine, University of the Republic, Montevideo, Uruguay). On day 100 of gestation, ewes were randomly divided into three groups (20 sheep in each one, called A, B and C).

From that moment until birth, the feeding of each group of ewes was supplemented with a mix-concentrate, divided into two daily doses, at 06:00 and 18:00 h. Group A (n = 20) received 700 g of a ration rich in PUFA; group B (n = 20) was fed with 700 g of commercial ration but supplemented with fish oil (20 mL of Vita-Mega3®, Laboratory Uruguay S.A.; Montevideo, Uruguay; fish oil rich in n-3 PUFA), administered *per os* each day, at 06:00 h; and Group C or control group (n = 20) was given 700 g of the same commercial ration.

Immediately after birth, each group was subdivided into two equal groups, thus forming 6 groups. From this moment and until postpartum day 120, the following protocol was used: Group A1 (n = 10) was fed with 700 g of the diet rich in PUFA (continuing with the same ration with which it had been fed up to this moment), while Group A2 (n = 10) was fed on the natural pasture; Group B1 (n = 10) was fed with 700 g of the commercial mix-concentrate together with 20 mL of Vita-Mega3® (*per os*, every 24 h, 06:00 a.m.), just as it had been fed up to this moment, whereas Group B2 (n = 10) was fed on a natural pasture;

Group C1 (n = 10) was fed with 700 g of the commercial mix-concentrate, similarly to how it had been fed up to this moment, and Group C2 (n = 10) was fed on natural pasture during the same time period.

The ingredients, of vegetable origin, of the commercial ration were corn, sorghum, barley, wheat, rice, soybean meal, sunflower flour, wheat bran, rice bran, malt sprouts and molasses. The ingredients of the ration rich in PUFA were sorghum, wheat bran, sunflower meal, soybean meal and linseed. The composition of the rations used, the energy value and the percentage of fatty acids in the rations and of the supplement Vita-Mega3 are shown in Tables 1 and 2.

Table 1. Supplied food and chemical composition of the experimental concentrates fed during the trial.

Item.	Unit	Commercial Ration *	PUFA-Rich Ration *
Dry matter intake	kg	0.62	0.62
Maximum humidity	%	12.5	13.0
Minimum crude protein	G	86.8	99.2
Minimum ethereal extract	G	18.6	18.6
Maximum crude fiber	G	62.0	62.0
Maximum ash	G	43.4	43.4
Calcium	G	25.8	25.8
Phosphorus	G	9.4	9.4
Chlorine	G	4.4	4.4
Sodium	G	8.1	8.1
Vitamin A	IU	3000	10,000
Vitamin D3	IU	1600	2000
Vitamin E	mg	27.5	27.5
Cobalt	mg	0.6	0.6
Iron	mg	108.0	108.0
Zinc	mg	168.0	168.0
Iodin	mg	1.7	1.7
Manganese	mg	120.0	120.0
Metabolizable energy	kcal/kg	2500	2550

* Amount per day. Kg: kilograms, g: grams, mg: milligrams, IU: international units, kcal/kg: kilocalories/kilograms.

Table 2. Percentage of fatty acids in the rations administered to sheep and in the supplement with fish oil (Vita–Mega3).

Item	Commercial Ration	PUFA-Rich Ration	Fish Oil Supplement
Lipids	2.76	7.11	
Humidity	30.0	27.8	
LA; 18:2 n-6	25.73	42.84	8.87
ALA; 18:3 n-3	3.33	18.1	1.11
AA; 20:4 n-6	0.07	0.06	0.55
EPA; 20:5 n-3	0.52	0.7	6.41
DHA; 22:6 n-3	0.05	0.1	13.63

Arachidonic acid (AA); linoleic acid (LA); alpha-linolenic acid (ALA); polyunsaturated fatty acids (PUFA); docosahexaenoic acid (DHA); eicosapentaenoic acid (EPA).

2.2. Sampling and Analytical Procedures

At 90 days of age, lambs' muscular samples (sample 1) were obtained by biopsy of the *Longissimus lumborum* muscle. The lamb's posterior dorsal was shaved, then disinfected with 10% povidone–iodine (Laboratorios Biogénesis Bago, Argentina). The skin and muscle between rib 12 and 13 and transverse process of the 13th thoracic vertebra on the left side were anesthetized with 2% lidocaine (Xylo-Efa®, Efa Laboratories, Montevideo, Uruguay). Subsequently, a 2 cm incision was made parallel to the spinous process, and with the help of forceps and scissors, a muscle sample was obtained (approximately 2 g), which was

deposited on aluminum foil, identified, and immediately frozen in liquid nitrogen. Finally, the incision was sutured, and antibiotics were applied.

At 120 days of age, 6 lambs from each group, corresponding to a heavy lamb with milk teeth, less than 13 months and weighing between 34 and 45 kg, were slaughtered. This type is called "Cordero Pesado" or Heavy Lamb and corresponds to a category of animals of the Uruguayan Marketing Program of the Uruguayan Wool Secretariat (SUL, http://www.sul.org.uy/sitio/Cordero-Pesado, accessed on 12 March 2016). A sample of lamb (sample 2) of the same muscle (*Longissimus lumborum*) was obtained, which was conditioned in foil, identified and frozen immediately in liquid nitrogen.

Fatty acids contained in the muscle were extracted and methylated according to the method of Wachira et al. [13] and quantified with gas–liquid chromatography at the Faculty of Science, University of the Republic, Montevideo, Uruguay. Briefly, lipid was extracted in chloroform containing 100 mg 2, 6-di-tertbutyl- p-cresol/l as antioxidant, and then anhydrous sodium sulfate was added. The solvents were removed under N2 and the lipids hydrolyzed with 2M-KOH in methanol–water (1:1, v/v). The fatty acids were methylated with a solution of diazomethane in diethyl ether and quantified with gas–liquid chromatography equipped with a column of 50 m $\times$ 0.25 mm internal diameter (CP-Sil 88 WCOT, Chrompak Ltd., Welwyn Garden City, Herts, UK) and using He as the carrier gas. Each peak of fatty acid methyl ester was identified according to the retention times of commercial standards (Supelco37, FAME MIX analytical). Fatty acids were expressed as g/100 g of total fatty acids.

2.3. Statistical Analysis

Statistical analysis was carried out with Statistica 6.0 (Stat Soft Inc, Tulsa, OK, USA, 2001). Descriptive statistics were performed, determining the statistics of central tendency suitable for each variable. Inferential statistics: t-Student test was performed for comparison of two groups (dependent groups, independent groups with equal variances and independent groups with unequal variances) according to a previous case study by Fisher test. ANOVA with post-hoc Tukey was used to evaluate statistical differences for more than treatments. All values are presented as average $\pm$ standard deviation ($\chi \pm$ SD). Significant differences were considered when $p < 0.05$.

When analyzing the total amount of n-3 and n-6 PUFA, as well as the values of ALA, EPA, DHA, LA and AA present in the muscle of the lambs of each group (A, B and C), Samples 1 and 2 (S1 vs. S2) were initially compared (A1, B1 and C1), and subsequently, the values measured in the samples obtained from lambs born from ewes which were supplemented in postpartum (A2, B2 and C2). Subsequently, the samples (S1) obtained from each experimental subgroup were compared (supplemented until delivery vs. supplemented postpartum A1 vs. A2, B1 vs. B2 and C1 vs. C2), as well as the statistical study of the second samples (S2) of each of the subgroups (A1 vs. A2, B1 vs. B2, and C1 vs. C2).

3. Results

3.1. n-3 Polyunsaturated Fatty Acids (n-3 PUFA)

The values of n-3 PUFA, ALA, EPA and ADH in lamb muscles of the three experimental groups are shown in Table 3.

The samples obtained 90 days postpartum (S1) from the lambs of the A1 group, whose mothers consumed a diet rich in PUFA after calving, presented a higher percentage of PUFA when compared to control group lambs (C1) (7.13 $\pm$ 1.23 and 4.10 $\pm$ 0.85, respectively). Likewise, the lambs of group B1 (7.11 $\pm$ 1.36), whose mothers received a fish oil supplement after delivery, also presented a significant difference from the control group (C1).

The postpartum samples obtained at 90 days (S1) from group A1 (7.13 $\pm$ 1.23) presented a higher percentage of n-3 fatty acids, when compared with those from the same group at 120 days (S2) after childbirth (2.94 $\pm$ 0.17). In group B1, when comparing the samples obtained in S1 (7.11 $\pm$ 1.36) and S2 (3.73 $\pm$ 0.41), a statistically significant difference was also found.

Table 3. Values (mean ± SD) of n-3 PUFA, in muscle of lambs (g/100 g of fatty acids, in dry weight).

Fatty Acid	Group A				Group B				Group C			
	Group A1		Group A2		Group B1		Group B2		Group C1		Group C2	
	S-1	S-2	S-1	S-2	S-1	S-2	S-1	S-2	S-1	S-2	S-1	S-2
n-3 PUFA	7.13 ± 1.23 [1]	2.94 ± 0.17 [2]	4.61 ± 0.51 [3]	4.15 ± 0.80 [4]	7.11 ± 1.36 [5]	3.73 ± 0.41 [6]	4.75 ± 0.63 [7]	4.61 ± 0.59 [8]	2.98 ± 0.93 [9]	2.88 ± 0.47 [10]	4.10 ± 0.85	3.76 ± 0.72 [11]
ALA	2.39 ± 0.44 [13]	1.69 ± 0.11 [14]	1.66 ± 0.35 [15]	1.59 ± 0.33	2.45 ± 0.48 [16]	1.96 ± 0.05 [17]	2.10 ± 0.20 [18]	1.92 ± 0.22	1.42 ± 0.27 [19]	1.35 ± 0.35 [20]	1.44 ± 0.57 [21]	1.36 ± 0.65
EPA	1.06 ± 0.48 [22]	0.33 ± 0.08 [23]	0.79 ± 0.20	0.74 ± 0.36	1.52 ± 0.57 [24]	0.43 ± 0.12 [25]	0.85 ± 0.34 [26]	0.89 ± 0.60	0.53 ± 0.29 [27]	0.29 ± 0.13	0.61 ± 0.2	0.61 ± 0.33
DHA	0.78 ± 0.66	0.20 ± 0.02 [28]	0.41 ± 0.35	0.35 ± 0.10 [29]	0.81 ± 0.56	0.39 ± 0.07 [30]	0.57 ± 0.23	0.44 ± 0.10	0.42 ± 0.26	0.24 ± 0.04 [31]	0.57 ± 0.3	0.41 ± 0.17

Statistical differences found ($p < 0.05$). n-3 PUFA: [1−2] $p = 0.0003$; [1−3] $p = 0.002$; [1−9] $p = 0.000006$; [2−4] $p = 0.01$; [2−6] $p = 0.003$; [5−6] $p = 0.01$; [5−7] $p = 0.003$; [5−9] $p = 0.0001$; [6−8] $p = 0.0013$; [6−10] $p = 0.007$; [10−11] $p = 0.031$. ALA: [13−14] $p = 0.008$; [13−15] $p = 0.010$; [13−19] $p = 0.001$; [14−17] $p = 0.001$; [15−18] $p = 0.024$; [16−17] $p = 0.042$; [16−19] $p = 0.0009$; [17−20] $p = 0.008$; [18−21] $p = 0.024$. EPA [22−23] $p = 0.008$; [22−27] $p = 0.045$; [24−25] $p = 0.002$; [24−26] $p = 0.03$; [24−27] $p = 0.003$. DHA: [28−29] $p = 0.019$; [29−30] $p = 0.0009$; [30−31] $p = 0.001$. S-1: sample 1 at 90 days; S-2: sample 2 at 120 days; n-3 polyunsaturated fatty acids (n-3 PUFA); linolenic acid (ALA); docosahexaenoic acid (DHA); eicosapentaenoic acid (EPA); Group A: fed with 700 g of the diet rich in PUFA before birth; Group B: fed with 700 g of the commercial mix-concentrate together with 20 mL of Vita-Mega3® before birth; Group C: fed with 700 g of the commercial mix-concentrate; Group A1, B1 and C1: fed after birth the same as prepartum; Group A2, B2 and C2: fed only on natural pasture during the same time period.

In the samples obtained 120 days after parturition (S2), the lambs of group B1, whose mothers received a fish oil supplement, presented a higher percentage of n-3 fatty acids (3.73 ± 0.41) when compared with the lambs of group A1, lambs of ewes supplemented with PUFA (2.94 ± 0.17), and with the lambs of group C1 (2.88 ± 0.47).

3.1.1. Alpha-Linolenic Acid (ALA)

The fat samples obtained from the lambs at 90 days postpartum (S1) of group B1 presented a higher percentage of alpha-linolenic acid (ALA) (2.45 ± 0.48) than the samples obtained from the lambs of the control group (C1) at the same moment (1.42 ± 0.27). This same behavior was evidenced when comparing this fatty acid in samples of the lambs born to mothers fed with PUFA in the ration (A1) (2.39 ± 0.44) and those obtained from lambs of the control group (C1).

For all experimental groups, the ALA obtained in the sampling on day 90 after delivery (S1) (2.39 ± 0.44, 2.45 ± 0.48 and 1.42 ± 0.27 for the groups A1, B1 and C1, respectively) was significantly higher than that obtained in these same groups in the sampling on day 120 postpartum (S2) (1.69 ± 0.11, 1.96 ± 0.05 and 1.35 ± 0.35 for groups A1, B1 and C1, respectively).

3.1.2. Eicosapentaenoic Acid (EPA)

The fat present in the muscle biopsy obtained at day 90 after calving (S1) of the lambs whose mothers received a supplantation with postpartum fish oil (B1) and PUFA in the ration after calving (A1) presented a higher level of eicosapentaenoic acid (EPA) (1.52 ± 0.57 and 1.06 ± 0.48 for A1 and B1, respectively) when compared with that obtained from the C1 group (0.53 ± 0.29).

This fatty acid was significantly higher in samples obtained in the S1 (1.06 ± 0.48, 1.52 ± 0.43 and 0.53 ± 0.29 for A1, B1 and C1, respectively) when compared with the values obtained in the S2 sampling (0.33 ± 0.08, 0.43 ± 0.12 and 0.29 ± 0.13 for A1, B1 and C1, respectively).

3.1.3. Docosahexaenoic Acid (DHA)

Although the samples obtained from the fat of the lambs born from the ewes of the supplemented groups after parturition showed a higher level of DHA (0.81 ± 0.56 and 0.78 ± 0.66 for the groups B1 and A1, respectively) when compared with group C1 (0.42 ± 0.26), these differences were not statistically significant.

3.2. n-6 Polyunsaturated Fatty Acids (n-6 PUFA)

The percentages of polyunsaturated fatty acids n-6 (n-6 PUFA) measured in the Longissimus lumborum muscle of lambs from the three experimental groups (A, B and C) are shown in Table 4.

When analyzing the fat samples obtained from the Longissimus lumborum muscle of the lambs, it is evident that in the first sample obtained after parturition (S1), group A1, whose mothers received a postpartum supplementation with PUFA in the ration, showed the highest percentage of polyunsaturated fatty acids n-6 (n-6 PUFA) (9.86 ± 1.24). This group achieved significant differences at this time from group B1, whose mothers received fish oil supplementation after parturition (5.91± 1.54) and with the control group (C1) (4.10 ± 0.82). Likewise, in this same postpartum sampling (day 90), group B1 achieved a significantly higher percentage of polyunsaturated fatty acid n-6 than the control group (C1).

When analyzing the differences achieved between the two postpartum samples (S1 vs. S2) in the three experimental groups, only group A1 showed a significant difference (9.86 ± 1.24 vs. 5.52 ± 0.79 for S1 and S2, respectively).

Table 4. Values (mean ± SD) of n-6 PUFA, in muscle of lambs (g/100 g of fatty acids, in dry weight).

Fatty Acid	Group A				Group B				Group C			
	Group A1		Group A2		Group B1		Group B2		Group C1		Group C2	
	S-1	S-2	S-1	S-2	S-1	S-2	S-1	S-2	S-1	S-2	S-1	S-2
n-6 PUFA	9.86 ± 1.24 [1]	5.52 ± 0.73 [2]	6.21 ± 0.39 [3]	6.82 ± 1.61 [4]	5.91 ± 1.54 [5]	4.82 ± 0.65	5.86 ± 1.13	5.73 ± 0.79 [6]	4.10 ± 0.82 [7]	4.21 ± 0.75 [8]	4.75 ± 0.57 [9]	4.64 ± 0.51 [10]
LA	5.6 ± 1.20 [11]	3.71 ± 0.11 [12]	3.95 ± 0.12 [13]	3.45 ± 0.51 [14]	3.94 ± 0.65 [15]	3.09 ± 0.82	3.03 ± 1.01	2.9 ± 0.97	2.72 ± 0.42 [16]	2.58 ± 0.79 [17]	3.09 ± 0.87	2.85 ± 0.96
AA	2.24 ± 0.74 [18]	0.86 ± 0.29 [19]	1.35 ± 0.47 [20]	1.03 ± 0.32	1.59 ± 0.47 [21]	0.99 ± 0.59	1.14 ± 0.19	1.18 ± 0.26	0.89 ± 0.36 [22]	0.57 ± 0.12 [23]	1.21 ± 0.42	1.12 ± 0.25 [24]

Statistical differences found ($p < 0.05$). n-6 PUFA: [1−2] $p = 0.0012$; [1−3] $p = 0.0004$; [1−5] $p = 0.000006$; [1−7] $p = 0.000002$; [2−8] $p = 0.012$; [3−9] $p = 0.0004$; [4−10] $p = 0.019$; [5−7] $p = 0.029$; [6−10] $p = 0.0017$[11−13]. LA: $p = 0.020$; [11−15] $p = 0.009$; 11-16 $p = 0.0013$; [12−17] $p = 0.001$; [13−14] $p = 0.003$; [15−16] $p = 0.003$. AA: [18−19] $p = 0.0074$; [18−20] $p = 0.03$; [18−22] $p = 0.002$; [21−22] $p = 0.016$; [23−24] $p = 0.0007$. S-1: sample 1 at 90 days; S-2: sample 2 at 120 days; n-6 polyunsaturated fatty acids (n-6 PUFA); arachidonic acid (AA); linoleic acid (LA). Group A: fed with 700 g of the diet rich in PUFA before birth; Group B: fed with 700 g of the commercial mix-concentrate together with 20 mL of Vita-Mega3® before birth; Group C: fed with 700 g of the commercial mix-concentrate; Group A1, B1 and C1: fed after birth the same as prepartum; Group A2, B2 and C2: fed only on natural pasture during the same time period.

3.2.1. Linoleic Acid (LA)

Samples obtained at 90 days postpartum from group A1 (5.6 ± 1.20), whose dams were supplemented with PUFA in the postpartum ration, had a significantly higher percentage of linoleic acid (LA) than those from group B1 lambs (3.94 ± 0.65) and group C1 (2.72 ± 0.42). Samples from group B1 lambs also had a significantly higher percentage of LA when compared to group C1 at the same time.

Group A presented significant differences between samples obtained at 90 (S1) and 120 (S2) days postpartum, both for those ewes that received a supplement with PUFA in the ration only until lambing (Group A2, 3.95 ± 0.12) and for those that continued with this diet after lambing (Group A1, 3.45 ± 0.51).

3.2.2. Arachidonic Acid (AA)

Arachidonic acid (AA) determined in the fat samples obtained at 90 days postpartum (S1) from lambs in the A1 group (2.24 ± 0.74) was significantly higher than that obtained from the fat of lambs in the C1 control group (0.89 ± 0.36). At the same time the lambs of group B1 (1.59 ± 0.47) showed a significantly higher percentage of this fatty acid when compared to the lambs of the control group (C1).

When comparing the percentages of AA between the two postpartum samples (S1 vs. S2) in the three experimental groups, only group A1 showed significant differences (2.24 ± 0.74 vs. 0.86 ± 0.29 for samples S1 and S2, respectively).

4. Discussion

There is information that shows that through the feeding of animals, the content of the different fatty acids present in the meat can be modified [2], altering the proportions between them, to make meat healthier, also with effects on aroma, taste and preservation [2,3,20–22]. This coincides with the results that we have obtained, taking into account the important circumstance that in our trial, diets were not fed directly to lambs but to their mothers during the last third of gestation and during lactation. Although in some experiments it is claimed that animal behavior, lamb yield and carcass traits were not affected by the diet composition of ewes [15,22], Ponnampalam et al. (2002) [3] and Gallardo Garcia et al. (2013) [1] asserted that changes in the fatty acid profile present in the milk fat produced by ewes could bring about changes in the characteristics and composition of the fat of the carcass of their lambs, offering the possibility to improve their quality, both from a nutritional and functional point of view.

The results derived from our experimental trial confirm a variation in the levels of n-3 fatty acids in the muscular fat of the lambs in relation to the feeding that their mothers received during gestation and the beginning of lactation. Thus, those ewes that consumed a food rich in PUFA were those that presented lambs with higher values of n-3 in muscle, followed by the lambs of those females who received a ration supplemented with fish oil. All of this coincides with findings of Wachira et al. [13] and Martínez Marín [20], who doubled the proportion of n-3 fatty acids in the *Longuissimus lumborum* muscle, when animal feed was supplemented with linseed, although fish oil also caused an increase in n-3 fatty acids when added to lamb diets [13,17,23]. Supplementary sources of fat with a higher content of n-3 PUFA included linseed meal (with high amount of linolenic acid), fishmeal, fish oil and marine algae (rich in EPA and ADH) [2,5,24–26]. According to Mateos et al. [27], linseed is especially rich in alpha-linolenic acid (ALA, 51%) and linoleic acid (LA, 13%). Due to this, the incorporation of linseed, flax flour or linseed oil into the diet [13–17,20,22,28] allows for increasing the content of alpha-linolenic acid in calf intramuscular fat and of the lambs [13]. Specifically, lambs fed with diets containing linseed practically doubled the ALA content of adipose tissue, increasing in the *Longissimus lumborum* from 1.4 in controls to 3.1 in lambs supplemented, while levels of EPA and ADH were multiplied by three with the inclusion of fish oil [13].

However, when evaluating linoleic acid (LA), we found that the highest percentages were found in the muscle of those lambs born from females receiving a diet supplemented

with fish oil. These findings coincide with different researchers who reported that fish oil has generally been a successful method of increasing CLA (conjugated linoleic acid) in meat [13,28]. For multiple investigators, the most effective measure to increase the percentage of EPA and ADH in meat of ruminants has been the incorporation of supplements rich in these fatty acids [1,2,12,13,15,20] into the diet, which coincides with our results, being that fish oil was the source of fat that contributes more EPA and DHA to the muscles of the lambs through the lipid supplementation to their mothers. Therefore, the highest percentages of DHA measured in the lamb muscle were found in those lambs born from ewes supplemented with fish oil, and with slightly lower values in the lambs whose mothers received a ration rich in PUFA, although it is more positive than the control group, which was fed only with a commercial concentrate. These results are similar to those reported by Wachira et al. [13], who supplemented the diet with fish oil and linseed, obtaining better results in animals fed with fish oil than in those fed with linseed.

Regarding the effect of ewe feeding on the yield and fatty acid composition of their lambs, Manso et al. [14] have shown that arachidonic acid (20: 4n-6) and eicosapentaenoic acid (20: 5-n-3) levels are similar in milk and subcutaneous fat but found higher concentrations of these fatty acids in intramuscular fat. In their opinion, this is because AA (20: 4n-6) and EPA (20: 5n-3) are mainly incorporated into the muscle phospholipid fraction, whereas n-3 fatty acids are found in amounts much lower in adipose tissue [29].

Brito et al. [30] and Montossi et al. [23] indicated that the muscle tissue of animals exclusively finished with grass contains higher percentages of n-3 fatty acids, whereas the muscles of animals finished with concentrates contain a higher percentage of n-6. These differences are justified by the fatty acid composition of the diet. Thus, grains contain more than 50% of total fatty acids as linoleic acid (LA), while forages show higher values of linolenic acid (ALA). In our trial, we have verified a similar behavior in the control group, in which no fat supplementation was added.

Thus, the amount of n-3 fatty acids present in the *Longissimus lumborum* muscle of lambs whose mothers were supplemented with PUFA and with fish oil was approximately 2.5 times higher than the amount detected in the same muscle in the lambs of the control group; this circumstance is similar to that reported in kids born from supplemented mothers, although with two-fold differences [7].

From a nutritional point of view, the ratio of n-6 and n-3 in foods recommended for human consumption should be less than 4 [13,31]. As a consequence, the incorporation of sources of PUFA such as fish oil and linseed in the ewes' ration enables the composition of the muscle fat of the lambs to be improved nutritionally, reducing the ratio n-6: n-3 in meat [1,13,14,22]. In the present study, we have verified that the PUFA: saturated fatty acid ratio may benefit from the inclusion of fish oil supplementation or a ration of linseed in the diet of pregnant ewes, with a ratio of 0.83/1 and 1.38/1, respectively.

The best n-6 values were found in the muscle fat of sheep fed with a PUFA-rich diet, which coincides with the experiences of Wachira et al. [13], who obtained higher amounts of LA in the group of lambs fed with linseeds, while finding lower values in those fed with fish oil.

It should be noted that the inclusion of fish oil in the diet leads to a depression of feed intake and therefore growth rate, although without affecting the rate of feed conversion [13]. In addition, free fats, especially those rich in PUFA, cannot be used at high doses, because they can have adverse effects on certain species of ruminal bacteria, which has a negative impact on ruminal digestion [20] and, particularly, on the fermentation of the fiber. This cellulolytic microflora of the rumen is the main responsible for biohydrogenation, limiting the ruminal production of both CLA, vaccenic acid and rumenic acid, reducing therefore the amount available for the tissues [1,2,31,32].

In line with other investigations, here, we found support for the possibility of implementing a management system to produce lamb meat with a better composition of fatty profile, improving its qualities to achieve possible beneficial effects for human health.

5. Conclusions

The type of PUFA supplement received by the ewes in the final stage of pregnancy and beginning of lactation had a favorable influence on the lipid profile of the meat of their lambs, increasing the concentration of n-3 and n-6 PUFA. Feeding ewes with a PUFA-rich diet or fish oil supplementation produced a change in the AL/ALA ratio in the intramuscular fat of their lambs, improving the profile of these fatty acids. In addition, all PUFA measured in the intramuscular fat of the lambs born from the supplemented ewes showed lower values at 120 than at 90 days of lamb life.

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