



Polyunsaturated fatty acid supplementation during breeding in nulliparous Katahdin ewes: reproductive efficiency and pre-weaning growth in lambs



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Abstract:

A total of 46 nulliparous Katahdin ewes were distributed under a completely randomized block design in two dietary treatments (n=23) around mating to evaluate the effects of adding of polyunsaturated fatty acid (PUFA) on the reproductive performance and pre-weaning growth in lambs. Treatments were two concentrates formulated isoenergetic and isoproteic that contained or did not contain (control) n-6 PUFA, and both were offered for 42 d (7 d before the breeding period and 35 d of breeding period; exposure to ram). Dietary addition of PUFA shortened ($P<0.05$) the estrus interval, without affecting ($P>0.05$) other reproductive variables. Lamb birth weight did not change ($P>0.05$) with addition of PUFA; however, the dietary addition of PUFA increased ($P<0.05$) pre-weaning growth rate and weaning weight in male lambs but not female lambs. Dietary addition of PUFA also improved ($P<0.05$) pre-weaning growth in twin birth lambs but not in single birth lambs. In conclusion, the inclusion of omega-6 PUFA in the diet of nulliparous Katahdin ewes during breeding is a promising dietary strategy since it shortens estrus interval without affecting other reproductive variables, and improves pre-weaning growth in male lambs and twin birth lambs.

Key words: Hair sheep, Linoleic acid, Flushing effect, Fertility, Postpartum growth.

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Because of their higher incidence of silent, shortest and less intense estrus, young ewes have lower reproductive efficiency than adult ewes⁽¹⁾. In addition, offspring born to young ewes also tend to exhibit low birth weight and slow pre-weaning growth⁽²⁾. Omega 6 (n-6) polyunsaturated fatty acid (PUFA) are essential for ruminants. They favor the reproductive processes of folliculogenesis, ovulation and estrus activity by stimulating synthesis of 17β -estradiol in ovaries, as well as prostaglandins $F2_\alpha$ ($PGF2_\alpha$) in endometrium^(3,4). Additionally, PUFA can regulate epigenetic processes and modify fetal programming during the early gestation, causing long-term postnatal effects in offspring⁽⁵⁾. So, formulation of breeding diets to include n-6 PUFA is a potential nutritional strategy to improve reproductive efficiency in nulliparous ewes and growth in their offspring.

In multiparous Pelibuey ewes, dietary supplementation with corn oil (rich in n-6 PUFA) improves follicular development, corpora lutea count and oocyte quality⁽⁶⁾, as well as embryo development⁽⁷⁾. This is attributed to increases in serum concentrations of cholesterol, insulin, 17β -estradiol, and progesterone⁽⁸⁾. However, the effects of including n-6 PUFA in the diet of mating ewes on reproductive variables has been inconsistent across studies^(9,10). In

multiparous wool breed ewes, n-6 PUFA supplementation around conception effectively reduced pre-weaning mortality and increased offspring capacity for post-natal thermoregulation⁽¹¹⁾. Note that these beneficial effects of n-6 PUFA have not been demonstrated in primiparous hair sheep. Therefore, the objective of this study was to evaluate the effect of adding n-6 PUFA in the breeding diet on the reproductive efficiency of nulliparous Katahdin ewes during the natural reproductive season, as well as on pre-weaning growth of their offspring.

The study was carried out during the sheep reproductive season (October-November) at the “El Tilzapote” ranch, located in the town of Ayutita, Autlán de Navarro municipality, Jalisco, Mexico (19°48’ N, 104°24’ W; 1,013 m asl). Experimental animals were 46 nulliparous Katahdin ewes with 9-mo of age, 43.9 ± 2.5 kg of live weight (LW) and 3.1 ± 0.6 units of body condition score (BCS) on a 1-to-5 scale⁽¹²⁾. Selection criteria for ewes were BCS from 2.5 to 3.5 units, and presence of signs of estrus one month prior to beginning of study, to ensure ovarian cyclicity.

Ewes were assigned to one of two dietary treatments (n = 23) following a completely randomized block design (CRBD; blocking factor= LW). Treatments consisted of feeding the ewes with corn silage (without grains) *ad libitum* and 400 g/d/ewe of a concentrate formulated with (PUFA group) or without (control group) a n-6 PUFA-rich source (Table 1).

Table 1: Chemical composition of concentrates and corn silage offered to ewes during the breeding period

Chemical components	Concentrates		Silage	Fatty acids, %	Concentrates	
	Control	PUFA			Control	PUFA
Crude protein, %	38.4	39.0	7.2	C14:0	0.5	0.1
Ash, %	6.0	5.0	25.7	C16:0	20.4	12.1
Crude fiber, %	10.2	10.1	11.6	C16:1 n-7	0.4	0.2
ADF, %	16.4	15.5	23.9	C18:0	3.3	3.6
NDF, %	53.0	43.5	59.1	C18:1 n-9	39.5	24.4
TDN, %	84.0	85.0	47.4	C18:2 n-6	28.0	50.3
DE, Mcal/kg DM	3.7	3.7	2.1	C20:0	0.4	0.3
ME, Mcal/kg DM	3.0	3.1	1.7	C18:3 n-6	0.3	0.2
				C20:1 n-9	0.4	0.1
				C18:3 n-3	2.8	2.4
				C22:0	0.1	0.2
				Polyunsaturated	31.4	53.2
				Monounsaturated	40.3	24.7
				Saturated	24.3	16.0

ADF= acid detergent fiber; NDF= neutral detergent fiber; TDN= total digestible nutrients; DE= digestible energy; ME= metabolizable energy.

Both concentrates were isoenergetic and isoproteic, although the PUFA concentrate contained 3% of a commercial vegetable oil rich in n-6. The control concentrate contained 3% frying oil, rich in oxidized fatty acids, to ensure that concentrate consumption was unaffected by differences in texture or consistency. Overall, ewes were fed the treatment diets during 42 d, 7 days before breeding and the 35 d of breeding period. The concentrates were offered once daily at 0700 h, before the silage was provided.

Once the breeding period ended, the ewes in both groups (PUFA and control) were fed corn silage for up to 4 wk before the lambing. During the last 4 wk of gestation and lactation period, both groups were also fed a concentrate at 500 g/d/ewe (metabolizable energy [ME]= 3.0 Mcal/kg dry matter [DM], and crude protein [CP]= 380 g/kg DM). This concentrate contained 50% soybean meal, 23% ground canola, 19% ground corn, 4% minerals, 1% urea and 3% oil. On d 8 and 90 (weaning) postpartum, the offspring had free access to creep feeding (CP= 210 g/kg DM, and ME= 3.5 Mcal/kg DM), which was formulated with 30% soybean paste, 63% corn, 2% gluten, 2% oil, 2% minerals and 1% milk substitute.

During the experiment, the ewes were penned separately by treatment (PUFA and control). The trough space within each pen was sufficient to ensure that all females could consume concentrate and silage simultaneously, without competition.

Feed samples were collected once a week from each group and processed for determination of bromatological analysis (DM, CP, ash and crude fiber)⁽¹³⁾, as well as neutral and acid detergent fiber fractions⁽¹⁴⁾. Total digestible nutrients (TDN)⁽¹⁵⁾, digestible energy (DE) and ME⁽¹⁶⁾ were calculated using formulas. Fatty acid profiles of concentrates were determined with a gas chromatographer (HP 6890, USA) equipped with an automatic injector (HP 7683, USA), tray with autosampler and a 100 m x 0.25 mm x 0.2 µm (film) capillary column at 29 psi (Supelco SP[®] 2560, USA).

Evaluation of corporal status: Ewe LW and BCS were recorded at d 7 before breeding, at the end of breeding and at parturition. Total weight gain (TWG) and daily weight gain (DWG) were calculated during the 42-d test period.

Evaluation of reproductive performance: Ewes did not receive a hormonal treatment prior to or during the breeding period, so the evaluation of the estrus activity was in accordance with the natural estrous cycle. Ewe estrus activity was recorded daily (0800 and 1800 h) during the 35-d breeding period (October 8 to November 11) using three Dorper breed rams with proven fertility. The rams were placed individually in the pens, but rotated between treatments. An ewe was considered to be in estrus when receptive to the ram, accepting natural mounting behavior without a movement reflex. The date and hour of the day of estrus detection was recorded for each ewe. Those in estrus were marked on the back with crayon and kept in the same pen. The number of mounts per ewe was not controlled, but a ratio of

15 females per male was considered sufficient to ensure pregnancy⁽¹⁷⁾. One month after ended the breeding period, a pregnancy diagnosis was done abdominally using a portable ultrasound (Model DP-10 Vet, Mindray, Shenzhen, China) equipped with a micro-convex transducer. Nonpregnant diagnosed ewes were removed from the pen. At parturition, ewes were kept in the same pen, recording only lambing date and type (single or twin).

The collected data were used to calculate the reproductive variables. Percentage of ewes in estrus was calculated by expressing the number of ewes in estrus as a percentage of the total number of treated ewes. Estrus interval was calculated as the number of days elapsed between the first day of breeding and the day with signs of estrus. Pregnancy rate was the percentage of ewes diagnosed as pregnant from the total number of mated ewes. Lambing rate was the percentage of ewes lambing from the total number of pregnant ewes. Fertility rate was the percentage of ewes lambing from the total number of treated ewes, and fecundity was the percentage of lambs born per mated ewe. Prolificity was calculated as the number of lambs born per ewe lambing. Finally, the percentage of ewes with single or twin lambing was calculated from the total number of ewes lambing.

Evaluation of pre-weaning growth: All lambs were identified at birth with metal ear tags, and their sex and birth weight were recorded. Lambs were weighed again at weaning (90 d) to calculate TWG and DWG.

Statistical analysis: All data were analyzed using the SAS statistical package⁽¹⁸⁾. Body status variables, estrus interval and prolificacy were evaluated with an analysis of variance under a CRBD. Variables expressed in percentage were analyzed with a Chi-squared test. Birth weight and lamb pre-weaning growth variables were also processed with an analysis of variance. However, a 2³ factorial arrangement in a completely randomized design was applied, where the model considered the fixed effects of dietary treatment (control and PUFA), sex (female and male), lambing type (single and twin) and all possible interactions. For pre-weaning growth variables, birth weight was added to the model as a covariate. Means were compared with a Tukey test at $\alpha=0.05$. The treatment \times sex \times lambing type interaction was not significant ($P>0.05$), so it was not included in results.

Ewe body status variables (LW, BCC, TWG and DWG) were unaffected ($P\geq 0.31$) by the dietary inclusion of PUFA at all measurement times (Table 2). Additionally, PUFA supplementation reduced ($P=0.05$) the estrus interval by 7 d, but did not affect ($P\geq 0.28$) other reproductive variables (Table 3). The estrus distribution results (Figure 1) showed that most of the ewes in the PUFA treatment ($P<0.05$) exhibited estrus in the first week of breeding (17.4 vs 47.8 %), while most of the control ewes ($P<0.05$) exhibited estrus in the second week (43.5 vs 17.4 %). On weeks 3, 4 and 5, the proportion of ewes in estrus decreased in both groups, although the control ewes had higher ($P<0.05$) percentage of estrus than those PUFA ewes on the wk 4.

Table 2: Live weight, weight gain and body condition in nulliparous Katahdin ewes fed either a concentrate containing n-6 polyunsaturated fatty acids (PUFA) or one without (Control)

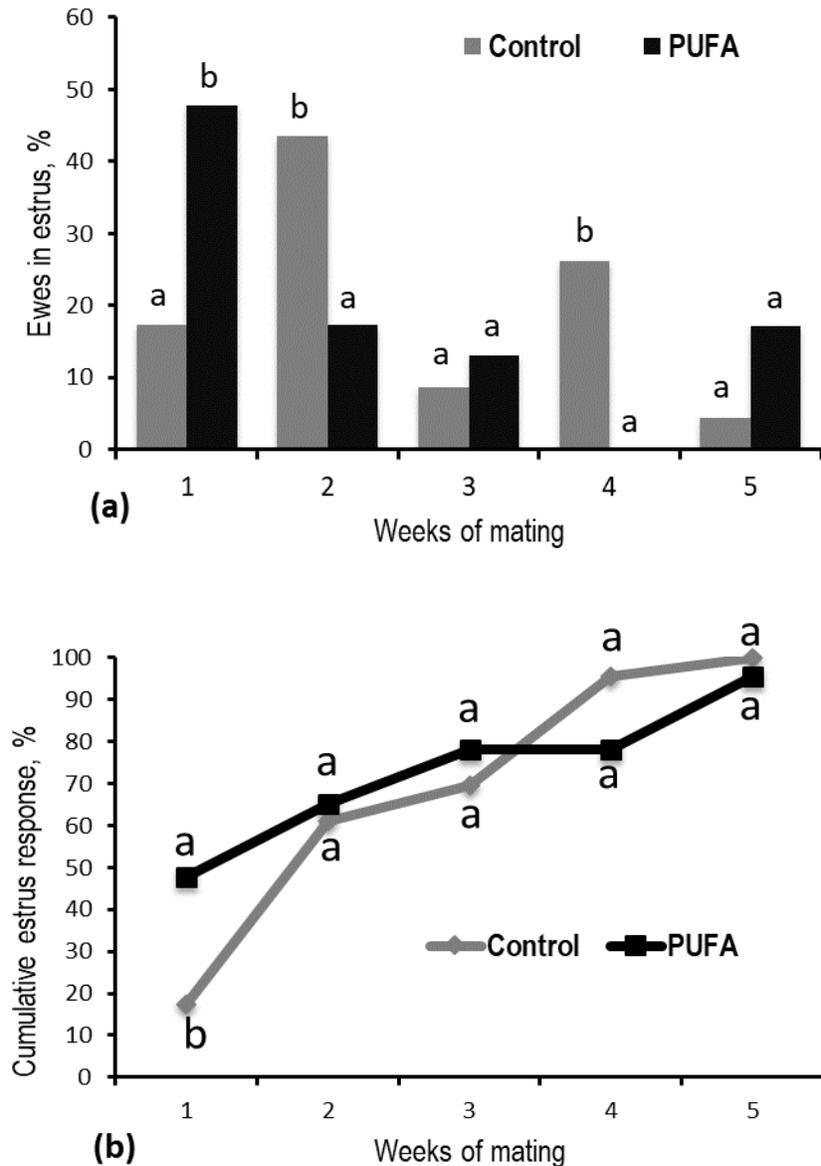
Variables	Groups		SE	P
	Control	PUFA		
Live weight, kg				
Initial (7 days pre-mating)	44.0	43.8	0.1	0.42
End of mating	49.5	48.6	0.6	0.31
Parturition	49.5	48.7	3.7	0.84
TWG, kg	5.6	4.8	0.6	0.37
DWG, g/day	132	114	14	0.37
Body condition				
Initial (7 days pre-mating)	3.1	3.2	0.1	0.52
End of mating	3.5	3.6	0.1	0.42
Parturition	3.3	3.2	0.2	0.50

SE= standard error; TWG= total weight gain; DWG daily weight gain.

Table 3: Reproductive performance in nulliparous Katahdin ewes fed either a concentrate containing n-6 polyunsaturated fatty acids (PUFA) or one without (Control)

Variables	Group		P
	Control	PUFA	
Ewes, n	23	23	-
Ewes in estrus, %	23/23 (100.0)	22/23 (95.6)	0.31
Estrus interval, days	17.0 ± 2.3	10.3 ± 2.3	0.05
Gestation rate, %	18/23 (78.3)	17/22 (77.3)	0.66
Birth rate, %	16/18 (88.9)	16/17 (94.1)	0.57
Fertility, %	16/23 (69.6)	16/23 (69.6)	1.00
Fecundity, %	24/23 (104.3)	21/23 (91.3)	0.35
Single births, %	8/16 (50.0)	11/16 (68.7)	0.28
Twin births, %	8/16 (50.0)	5/16 (31.2)	0.28
Prolificity, n	1.5 ± 0.1	1.3 ± 0.1	0.67

Figure 1: Estrus distribution per week (a) and cumulative weekly estruses (b) during mating period in nulliparous Katahdin ewes fed either a concentrate containing n-6 polyunsaturated fatty acids (PUFA) or one without (Control)



^{a,b} Different letters in the same week indicate differences between groups ($P < 0.05$).

Pre-weaning growth results are shown in Table 4. The treatment \times sex interaction did not affect ($P > 0.11$) birth weight, but did affect ($P < 0.05$) DWG, TWG and weaning weight. Dietary inclusion of PUFA increased ($P < 0.05$) TWG, DWG and weaning weight in male lambs, but not in female lambs. The treatment \times lambing type interaction affected ($P < 0.05$)

birth weight and pre-weaning growth variables. Lambs born from single lambing had higher ($P<0.05$) birth weight than those born from twin lambing in both the dietary groups. In contrast, the PUFA treatment increased ($P<0.05$) DWG, TWG and weaning weight in lambs born from twin lambing, but not ($P>0.05$) in single birth lambs. Finally, the lambing type \times sex interaction affected ($P<0.05$) birth weight, but not TWG, DWG and weaning weight. Overall, birth weight was higher ($P<0.05$) in single birth lambs than in twin birth lambs in both sexes.

Table 4: Pre-weaning growth in lambs of Katahdin ewes fed either a concentrate containing n-6 polyunsaturated fatty acids (PUFA) or one without (Control)

Study Factors			Growth Variables			
Factor A	Factor B	n	LWB (kg)	LWW (kg)	TWG (kg)	DWG (g/day)
Treatment \times sex interaction						
Control	Female	14	3.8 \pm 0.2 ^a	28.0 \pm 1.2 ^a	24.2 \pm 1.2 ^a	269 \pm 14 ^a
	Male	10	3.9 \pm 0.3 ^a	28.2 \pm 1.7 ^a	24.5 \pm 1.7 ^a	276 \pm 19 ^a
PUFA	Female	15	3.9 \pm 0.2 ^a	28.5 \pm 1.2 ^a	24.7 \pm 1.2 ^a	278 \pm 13 ^a
	Male	6	3.7 \pm 0.3 ^a	34.3 \pm 2.9 ^b	30.5 \pm 2.9 ^b	341 \pm 33 ^b
Treatment \times lambing type interaction						
Control	Single	8	4.5 \pm 0.3 ^a	29.6 \pm 2.0 ^{ab}	25.9 \pm 2.0 ^{ab}	306 \pm 20 ^a
	Twin	16	3.2 \pm 0.2 ^b	26.6 \pm 1.4 ^a	22.8 \pm 1.4 ^a	239 \pm 12 ^b
PUFA	Single	11	4.1 \pm 0.2 ^a	31.6 \pm 2.4 ^b	27.9 \pm 2.4 ^b	324 \pm 25 ^a
	Twin	10	3.5 \pm 0.2 ^b	31.1 \pm 2.3 ^b	27.4 \pm 2.3 ^b	295 \pm 26 ^a
Lambing type \times sex interaction						
Single	Female	14	4.5 \pm 0.2 ^a	29.8 \pm 1.5 ^a	25.2 \pm 1.5 ^a	281 \pm 13 ^a
	Male	5	4.2 \pm 0.3 ^a	32.0 \pm 2.7 ^a	27.9 \pm 2.7 ^a	310 \pm 29 ^a
Twin	Female	15	3.2 \pm 0.2 ^b	27.7 \pm 1.5 ^a	24.5 \pm 1.5 ^a	272 \pm 16 ^a
	Male	11	3.4 \pm 0.2 ^b	28.8 \pm 2.3 ^a	25.4 \pm 2.3 ^a	282 \pm 25 ^a

n= number of lambs; LWB= live weight at birth; LWW= live weight at weaning; TWG= total weight gain; DWG= daily weight gain.

^{ab} Different letter superscripts in the same column and same double interaction indicate differences ($P<0.05$).

Addition of n-6 PUFA in the breeding diet had no effect on body status in the Katahdin ewes, which was expected as both the PUFA and control concentrates were isoenergetic and isoproteic, with a relatively low (3%) oil inclusion level in both diets. Sheep diets should not be formulated with more than 6% of any vegetable oil because excessive oil intake can reduce ruminal microbial activity and, consequently, feed intake and body status⁽³⁾. Ewe LW and BCS results coincide with previous reports⁽¹⁰⁾.

Inclusion of n-6 PUFA in the breeding diet shortened the natural estrus interval by seven days in the Katahdin ewes. This may be due to two possible mechanisms, depending on which

estrus cycle stage a sheep is in when it begins the intake of a PUFA-rich diet. First, PUFA intake may have caused early lysis of the corpus luteum by stimulating PGF 2α synthesis, since these are precursors of arachidonic acid⁽⁴⁾. Second, the presence of PUFA could have increased growth and steroidogenic capacity in the pre-ovulatory follicles⁽⁸⁾. The shortened estrus interval observed in the present results coincides with a study of Merino ewes, which exhibited a shorter estrus interval when fed oleaginous seeds rich in n-6 PUFA during the breeding period⁽¹⁹⁾.

Feeding with n-6 PUFA during the breeding period helped to naturally synchronize estrus activity in the evaluated ewes. About 50 % of the ewes in the PUFA treatment presented estrus and were mated naturally within the first week of the breeding period. This has great practical relevance since feeding to ewes with PUFA can be applied to reduce production times associated with breeding, lambing, lamb pre-weaning care and general herd management.

Although the estrus interval was shorter by including n-6 PUFA in the breeding diet, it did not change reproductive performance in Katahdin ewes. This contradicts the expected results, based on previous studies. For example, previous studies in Pelibuey ewes indicate that n-6 PUFA supplementation during breeding favors follicular⁽⁶⁾ and embryonic⁽⁷⁾ development, as well as the ovulatory rate⁽⁷⁾ and corpus luteum functionality⁽⁸⁾. Hence, higher pregnancy rate, fertility, prolificacy and multiple lambing in nulliparous Katahdin ewes was expected. In contrast, in another study, the fertility and lambing rates, and the proportion of twin births, improved in multiparous Afshari ewes fed n-6 PUFA protected with calcium salt^(9,20). Supplementation with soybean oil as a n-6 PUFA source in Pelibuey, Blackbelly and Pelibuey x Blackbelly ewes, is reported to increase prolificacy but not the conception and fertility rates⁽¹⁷⁾.

The lack of improved reproductive performance in PUFA ewes may have been caused by the short (7-d) pre-breeding feeding time; it is required to offer the concentrate at least 20 d before exposing the ewes to the ram, and to continue during the breeding period^(9,17,20). Another possible cause is low post-intake PUFA bioavailability. Only 15 % of PUFA reach the intestine intact and ready for absorption, the remaining percentage will be saturated during ruminal biohydrogenation⁽³⁾. For example, ewes fed sunflower oil protected in calcium salt exhibited greater follicular growth, serum concentrations of steroidal hormones, fertility, prolificacy and lambing rates than those fed unprotected sunflower oil⁽²⁰⁾.

The effect of PUFA intake in ewes during breeding on offspring birth weight is inconclusive in the literature. Some studies report no effects⁽¹⁹⁾, while others indicate improvements^(9,20). In the present study, birth weight was unaffected by PUFA maternal intake and offspring sex, although lambing type did caused variation in this variable. Birth weight was higher in single

birth lambs than in twin birth lambs, regardless of sex and mother's origin; this is well documented in the literature⁽²¹⁾.

The PUFA treatment ewes exhibited better pre-weaning growth in lambs, particularly in male lambs and twin birth lambs. Fetal programming is known to begin post-conception, and varies by sex and lambing type⁽²⁾. Male fetuses are programmed to have better postnatal muscle development than female fetuses⁽²²⁾. Multiple gestation fetuses, unlike single gestation fetuses, are programmed to naturally have intrauterine growth restriction, which can negatively affect its postnatal development⁽²³⁾. The PUFA are associated with fetal programming of lipid and muscle metabolism, as well as neonate behavior and vigor⁽⁵⁾. The current findings therefore suggest that addition of n-6 PUFA exerts long-term beneficial effects on pre-weaning growth in lambs by modifying fetal programming in early gestation. Indeed, this nutritional strategy appears to partially reverse fetal growth programming in twin-pregnancy fetuses. This would explain the improved pre-weaning growth in twin birth lambs from PUFA treatment ewes. Future research is needed to confirm the possible effects of PUFA in fetal programming of hair sheep.

Overall, dietary addition of n-6 PUFA during the breeding period shortened the time of estrus onset in nulliparous Katahdin ewes, but did not improve overall reproductive performance during the natural reproductive season. It also had long-term beneficial effects on pre-weaning growth in male lambs and those born in twin births.

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