



Assessment of milk production in Assaf ewes born as twin based on correspondence analyses and mixed model

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ABSTRACT

The aim of this study was to determine whether the presence and number of male and female twins affected the future milk yields of female ewes in adulthood. The study was based on 10,049 milk production records from 12 dairy sheep farms in Spain. Total milk yield (TMY) was categorized as either Low or High, and daily milk yield (DMY) was categorized as either Low/1, Intermediate-low/2, Intermediate-high/3, or High/4. Twins were categorized as follows: (1) ewes born without a twin (0 T), (2) ewes born with at least one female twin (at least 1FT: 1 and 2 sisters), (3) at least one male twin (at least 1MT: 1 and 2 brothers), or (4) with one male and one female twin (1MT+1FT: one brother and sister). Correspondence analyses indicated that ewes in the 0 T group were associated with low TMY and DMY, and twin births (at least 1MT and 1FT groups) were associated with 2–4TMY levels, and 3–4DMY levels. Mixed-effect-model indicated that ewes in the at least 1MT group had a positive effect on TMY ($P < 0.05$) and DMY ($P < 0.01$), than did ewes in the 0 T group. In summary, litter size and litter sex ratio in intrauterine life had a positive effect on milk production in ewes in adulthood. This effect is positively evidenced in ewes born with at least one-male-twins. This information might be useful in identifying ways to improve the economic-productive efficiency of sheep dairy systems.

1. Introduction

Dairy sheep production systems have an important impact on social and economic development in European countries, which produce about 30% of the global sheep milk supply. Specifically, Spain, France, Italy, and Greece contribute 23% of the global dairy sheep production (FAOSTAT, 2022). Reductions in operating costs and optimal use of resources influence the productive efficiency of those systems at individual dairy sheep farms (Pérez et al., 2007). Furthermore, knowledge and management of both intrinsic (e.g., genetic and physiological stage of the animals) and extrinsic factors influence the performance of dairy sheep flocks (e.g., external-environmental factors; Hussein et al., 2016; Pollott and Gootwine, 2004; Thornton, 2010).

In addition, external factors include those that occur in the intra-uterine/prenatal stages and in the early stages of postnatal development, which can have positive or negative effects on internal organs and glands, and on future individual performance. That phenomenon is

known as “Developmental programming” (Sartori et al., 2022), which has been adopted in the livestock industry (Abuelo, 2020; Foxcroft et al., 2009; Fowden et al., 2010; Reynolds and Vonnahme, 2017). For instance, in dairy ewes, undernourishment in pregnant sheep had a negative effect on the milk production of their daughters (Paten et al., 2017).

In addition, studies have shown that foetal sex ratios among twin or triplet pregnancies can have a significant effect on offspring development. For instance, freemartin, a well-documented syndrome in cattle, is characterized by the presence of a female foetus that has a varying degree of intersexuality or masculinization, which is influenced by the placental vascular network between male and female foetuses (Padula, 2005). Freemartin is not as common in small ruminant species, likely because of limited clinical reporting within extensive production systems (Padula, 2005). It is possible that the sex of twins and triplets might affect the future development or productive performance of the opposite-sex foetus in sheep and goats, but it has not been investigated

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thoroughly. Observational studies (e.g., data collected in commercial dairy sheep farming) might be an effective means of obtaining insights into this issue (Bello et al., 2018). Furthermore, observational studies in this field might be helpful in identifying novel research lines aimed at designing strategies or facilitating decision-making to enhance the efficiency of dairy sheep systems.

The objective of this study was to investigate the relationship between the presence and number of male and female lambs during foetal life and milk production in Assaf dairy female lambs in their adulthood. This study was based on milking records that had been collected from Spanish commercial dairy sheep farms, which were analyzed by simple correspondence analyses and mixed-effect-model.

2. Materials and methods

2.1. Database construction and variable definition

The analysis was based on 10,672 milking records from 12 Assaf dairy farms in Castilla-Leon, Spain, which had been collected between 2002 and 2023. The farms were registered in the Spanish Assaf Sheep Farmer Association (ASSAFE). Data errors ($n = 623$) were excluded from the analysis; therefore, the analysis was based on 10,049 milking records.

Data included total milk yield (TMY), milking length (ML), and daily milk yield per lactation (DMY = TMY/ML). In addition, these variables were categorized as either Low or High at one of four levels (Low = 1TMY-1DMY, Intermediate-Low = 2TMY-2DMY, Intermediate-High = 3TMY-3DMY, and High = 4TMY-4DMY). TMY and DMY were categorized using ranks based on median and quantiles.

Other variables included were those associated with the number of male and female twins that shared the same uterus and the milk production of ewes in adulthood. Original data set included the following categories: (1) ewes born without twins (0 T; $n = 5073$; 50.5%), (2) ewes born with one female twin (1FT; $n = 2518$; 25.1%), (3) ewes born with two female twins (2FT; $n = 130$; 1.3%), (4) ewes born with one male twin (1MT; $n = 2086$; 20.8%), (5) ewes born with two male twins (2MT; $n = 77$; 0.8%), or (6) ewes born with one male and one female twin (1MT+1FT; $n = 165$; 1.6%). Because lower number of records in 2FT and 2MT groups number of male and female twins were regrouped and recodified to quantify the effects of the presence of at least one male and or female twin on the future milk production of their female twin. Thus, this explanatory variable (named SEX) included the following categories: (1) ewes born without twins (0 T; $n = 5073$; 50.5%), (2) ewes born with at least one female twin (at least 1FT; $n = 2648$; 26.4%), (3) ewes born with at least one male twin (at least 1MT; $n = 2163$; 21.5%), or (4) ewes born with born with one male and one female twin (1MT+1FT; $n = 165$; 1.6%). Age was reflected in the number of parturitions (NP2; first or two or more parturitions). Moreover, lambing season (LS) was also included.

2.2. Statistical analyses

All statistical analyses were performed in RStudio® (version 2024.04.2). Simple correspondence analyses (CA) were performed to quantify and visualize the association between litter sex ratios (SEX) and milk production levels (1–4TMY and 1–4DMY). Previously, a *Chi-square* Test was used to determine the dependence-assumptions between variables.

In our study, a mixed-effect-model was used exclusively to quantify the influence of the number of foetal male or female twins (SEX factor) on milk production (TMY and DMY) of the ewes in adulthood. Therefore, data from the 1MT+ 1FT group ($n = 171$) were excluded from those analyses. Other factors included in the model were ML (quantitative variable), LS, NP2 (categorical variable), lambing year (LYR) and FA (random effect). In addition, the assumption of collinearity was tested by variance-inflation factors ($vif < 5$). Significant intercept, Akaike and

Bayesian information criterion and coefficient determination (R-squared) were used to identify the best fitted model. The final models used for TMY and DMY included the following estimators:

$$TMY = \beta_0 + \beta_1 \times SEX_i + \beta_2 \times ML-s_j + \beta_3 \times NP2_k + \beta_4 \times LS_l + \beta_5 \times FA_m + \beta_6 \times LYR_n + \varepsilon_{ijklmn}$$

$$DMY = \beta_0 + \beta_1 \times SEX_i + \beta_2 \times NP2_j + \beta_3 \times LS_k + \beta_4 \times FA_l + \beta_5 \times LYR_m + \varepsilon_{ijklm}$$

where SEX = fixed effect of litter sex ratio (male or female twin), ML-s = fixed effect of scaled milking length (confounding variable), NP2 = fixed effect of number of parturitions (confounding variable), LS = fixed effect of lambing season (confounding variable), FA = farm random effect, LYR = lambing year random effect and, Error = effect attributed to error. Statistical significance and tendency were established at $P < 0.05$ and $P > 0.05$ and $P \leq 0.10$.

3. Results and discussion

Chi-square tests indicated that milk production level (1–4TMY and DMY) and SEX categories were significantly ($P < 0.001$) correlated, which was a requisite assumption for performing the CA. The CA visualization showed that singleton ewes (0 T) were more strongly associated with low milk production (1TMY and 1DMY) than were twin-born ewes (Fig. 1). Conversely, ewes that shared a litter with a male and a female twin (1MT+1FT) were more distanced with different DMY levels (Fig. 1b).

The mixed-effect-model confirmed and quantified the relationships identified by the CA. Specifically, the model demonstrated that ewes that had shared a litter with at least one male (at least 1MT) increased the TMY by 6.77 ($P < 0.05$) l/ewe than did singleton ewes (0 T; Table 1). Similarly, the fixed effect demonstrated that ewes that had shared a litter with at least one male twin (at least 1MT) increased the DMY by 0.05 l/d/ewe than did ewes in the 0 T group ($P < 0.01$; Table 1). Additionally, ewes that had shared with at least one female (at least 1FT) tended to increase the TMY ($P = 0.07$) and DMY ($P = 0.09$) compared with singleton ewes (0 T group; Table 1).

These results might be helpful in the development of management strategies or facilitate decision-making in the dairy sheep industry. Previous data analyses in our group demonstrated the positive sex-ratio litter effect on milk performance in Lacunae and Churra ewes (Abecia and Palacios, 2018). However, currently there are not previous information about the intrauterine effect of litter sex-ratio and litter size on future milk yield in female lamb. The selection of female lambs from twin litters and pure-male litters might be a practical strategy to improve productive indices in dairy sheep farms without requiring additional investment of time or money. The coefficients of determination (R-squared) for TMY (0.70) and DMY (0.41) indicated that the mixed model provided a better fit for TMY than it did for DMY. The positive effect of twins on TMY observed in our best-fitted model suggests a synergistic effect of litter size and sex-ratio on the future dairy performance of female lambs. Litter size is a biological trait that is influenced by genetic factors (Gootwine et al., 2008); however, it can be affected by extrinsic factors such as the type of reproductive management of flocks implemented by farmers (e.g., the combination of progestogens and eCG or high-energy diets (flushing) during the follicular phase; Simões et al., 2021), which are commonly used to increase ovulation rate and, consequently, prolificacy rate in small ruminants (Cueto et al., 2020).

In addition, the effect of the presence of twins on TMY identified in our model was modulated by others factors such as lamb age, number of parturitions (NP2), milking length (ML), lambing season (LS), flock management, and lambing year (FA, LYR; as random effect). NP2 and ML had a significant effect ($P < 0.001$, respectively; Table 1) in TMY model. Specifically, the estimates for NP2 - specifically for two or more parturition levels - from TMY model were similar to the results of Pollot and Gootwine (2004), who reported an increase in TMY after the second

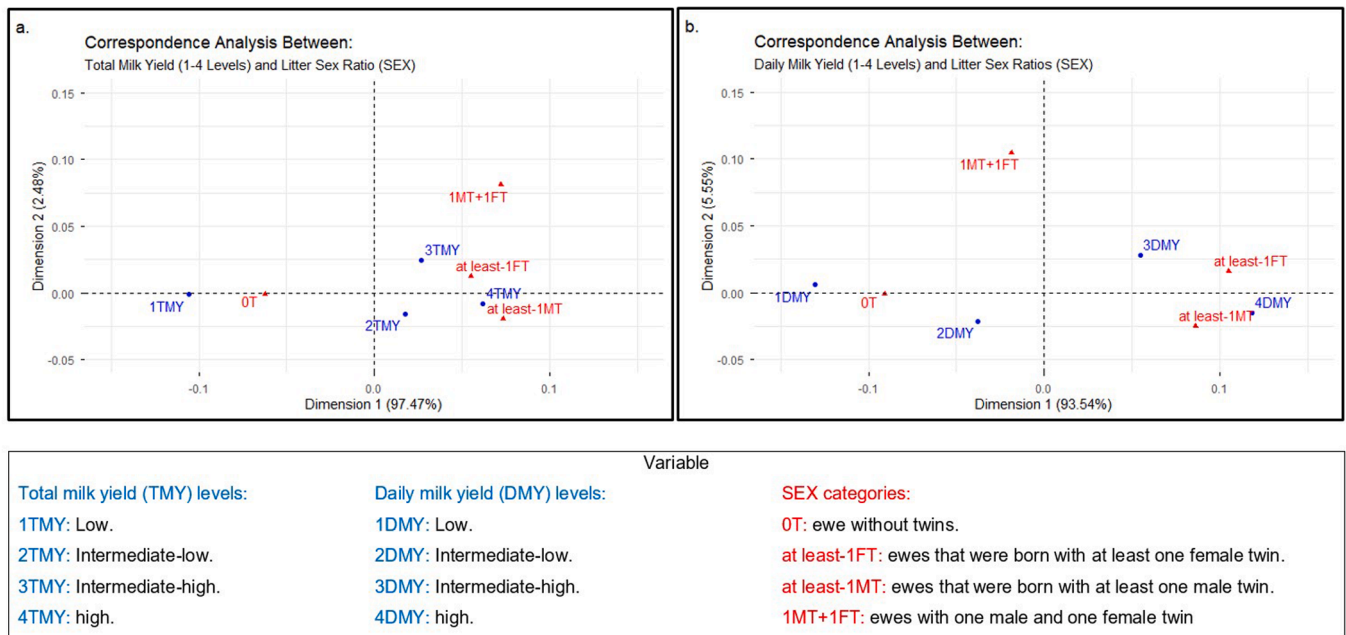


Fig. 1. Simple correspondence analyses between four levels of milk yield (Blue; a: total milk yield/TMY; b: daily milk yield/DMY) and presence/absence of male or female twins (Red; SEX) in Assaf dairy ewes. Dimension 1 (horizontal; X-axis) and 2 (vertical; Y-axis) illustrate the closer relationships between categorical milk yield levels and the presence or absence of male or female twin categories.

Table 1

Mixed model estimates from a linear mixed model for total (TMY) and daily (DMY) milk yield in Assaf ewes.

Predictors	TMY		P value	Estimates	DMY	
	Estimates	95% C.I.			95% C.I.	P value
Fixed effect						
(Intercept)	330.87	283.48 – 378.25	< 0.001	1.79	1.55 – 2.04	< 0.001
(SEX) 0 T	Reference					
(SEX) At least-1FT	5.09	-0.33 – 10.52	0.066	0.03	-0.00 – 0.06	0.090
(SEX) At least-1MT	6.77	0.89 – 12.64	0.024	0.05	0.01 – 0.08	0.006
ML (scaled)	2.12	2.08 – 2.15	< 0.001	-	-	-
NP2 [1 parturition]	Reference					
NP2 [\geq 2 parturition]	61.32	56.26 – 66.38	< 0.001	0.29	0.26 – 0.32	< 0.001
LS [Winter]	Reference					
LS [Fall]	-13.02	-21.77 – -4.27	0.004	-0.04	-0.09 – 0.01	0.100
LS [Spring]	3.83	-0.99 – 8.65	0.120	0.05	0.02 – 0.07	0.001
LS [Summer]	-8.18	-16.01 – -0.36	0.040	-0.02	-0.07 – 0.02	0.276
Random effect	Variance			Variance		
FA (Farm)	4688.28		68.47	0.13		0.36
LYR (Lambing year)	4035.82		63.53	0.11		0.33

n.s.: non-significant; *p < 0.05; ** p < 0.01; *** p < 0.001; C.I: confident intervals; 0 T: ewes born without a twin; At least 1FT/1MT: ewes born with at least one female or male twin; ML: milking length; NP2: number of parturitions (1 vs 2 or more parturition); LS: Lambing season; R² (TMY): 0.70; R² (DMY): 0.41; AIC/BIC (TMY): 120352/120352; AIC/BIC (DMY): 18212/18284.

and subsequent parturitions compared with first parturition in Assaf ewes under intensive management. A significant and positive higher effect of lambing season on TMY was also observed in fall (P < 0.05; Table 1). This finding is consistent with Abecia et al. (2017), who reported lower milk yield per day in September compared with February, April, and November in Assaf ewes.

Maternal nutritional state is an environmental factor that can influence milk yield. Paten et al. (2017) suggested that maternal weight and nutritional status in ewes during pregnancy influence mammary gland development and future milk production in their lambs; however, the lack of information on maternal nutritional status in our database suggests the need for further studies to measure its impact on twins and their association with TMY. Both the number and the sex of the offspring influence the mother’s milk production. The effect of offspring sex suggests the existence of prenatal mechanisms (e.g., fetal hormones) that modulate the secretory capacity of the mammary gland, in addition

to the well-established effects of litter size and early suckling stimulation (Abecia and Palacios, 2018), or an effect on the rearing of lambs, which may be better fed by their mothers due to increased milk production.

4. Conclusions

The results provide some evidence that female lambs born from twin births are more likely to have a high milk yield in adulthood than are those born from single births. In addition, the presence of additional male twins (twins and/or triplet lambing) during intrauterine life had a positive additive effect on the milk yield in adulthood than female-lambs from single parturitions. These findings provide a preliminary approach to assess the potential effects of male and female twin relationships during fetal life or during breastfeeding for expectant mothers on productive performance in adulthood. More studies on polytocous species might contribute to the development of management strategies that can

increase the efficiency of dairy sheep production systems.

CRedit authorship contribution statement

I. Contreras-Solís: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Abecia Jose Alfonso:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Conceptualization. **Palacios Carlos:** Writing – review & editing, Writing – original draft, Software, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that might have appeared to influence the work reported in this paper.

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