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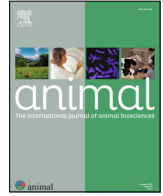
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Estimation of milk production in suckling mares and factors influencing their milk yield



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ABSTRACT

Research about mare's milk is mainly focused on quality and information about quantity is incomplete partly due to the lack of a consensus on the method of measuring milk yield. The live weight, body condition at foaling and age of mares are factors influencing milk yield. The influence of mare parity, however, remains unclear. Over a period of 2 years (2018–2019), milk yield was evaluated on 65 mares (51 multiparous and 13 primiparous). Mares and foals were kept in a group at pasture. One method of milk yield measurement and one proxy method were applied; milking and weight-suckle-weight (WSW), respectively. The procedure was performed at five timepoints during the lactation period (3–30–60–90 and 180 days) without repetition. The relevance of WSW was addressed by studying the correlation between the two methods on 23 individuals. Factors influencing milk yield, through milking data, were studied on 57 individuals. Data was divided into two subsets. The first was an explanatory matrix containing the live weight of mares 24 h after parturition, parity, age, year of lactation and foal gender. The second was a response matrix containing data from milking at the five timepoints of the lactation. A correlation was found ($RV = 0.41$) between milking and WSW at day 3, however no correlation was found for other timepoints ($RV \leq 0.15$). The live weight of the mare 24 h after foaling, age and parity appeared to have a significant impact on milk production ($P < 0.05$). Thus, older or multiparous mares showed a higher milk yield than younger or primiparous mares. In addition, mares with a higher live weight after foaling produced more milk than those with a lower live weight. Overall, results can lead us to two main conclusions. First, the WSW method performed at five different timepoints of the lactation, but without repeated measurements, is not an efficient way to estimate the milk yield of mares. Secondly, results concerning the live weight and age of mares were in accordance with previous studies. The influence of parity was also highlighted, confirming trends showed by other authors. Age and parity are closely related in our population, making it difficult to differentially assess their effects. Being able to identify the impact of both factors independently would benefit several sectors of the horse industry from sport to mare milk producers.

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Implications

Methods to be used to estimate milk production in the nursing mare are under discussion, with few studies assessing the impact of various factors on milk production. The possibility to use a proxy method (weigh-suckle-weigh), instead of direct measurement by milking, was studied, but this method was not reliable as neither

measurements were correlated. Live weight 24 h after foaling was correlated to milk production. Young primiparous produced less milk than older, multiparous mares. So far, only milking can provide a relevant estimation of milk yield as a standardised method if proposed.

Introduction

Milk yield is directly related to foal growth in horses for the first month of the foal's life (Schmidt et al., 2010). Nevertheless,

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although numerous studies have been carried out on milk quality, information about quantity remains scarce. In previous studies, milk yield was estimated as ranging between 2% and 3.5% of a mare's BW (Oftedal et al., 1983; Doreau, 1994), depending on the breeding objectives (nursing foals or dairy mares). Moreover, body condition at foaling is important for subsequent milk production as very thin mares with a body condition score (BCS) $BCS < 1.5$ (French scale of 1–5) produce less milk than mares with a $BCS > 3.5$ (Doreau et al., 1993). The effect of nutrition on milk yield remains unclear as some authors have demonstrated the lack of effect of dietary supplementation (Doreau et al., 1988; Martin et al., 1992) whereas, others show the contrary (Gibbs et al., 1982; Doreau et al., 1992). In horses, nutritional status can be estimated through bodyweight. Successive BCS estimations are mainly used to evaluate variations in fat deposits while BW provides an overall evaluation of changes in body mass, that can be related to fat but also to muscle mass variations (Carter and Dugdale, 2013). These two indicators, however, are correlated with a weight gain or loss of 10% for each point under or over a BCS of 3, respectively (Martin-Rosset, 2015).

In addition the effects of nutrition on the lactation of mares, Doreau and Martuzzi (2006) have highlighted the effect of the age of the mares, nursing mares reaching maximum milk yield when aged from 8 to 11 years. The available results are few and contradictory. Gibbs et al. (1982) demonstrated an effect of parity with primiparous producing less milk than multiparous mares (respectively, 10.4 and 11.7 kg of milk per day) whilst only a trend or no effect was found by others (Doreau et al., 1991; Trottier et al., 2006). Nevertheless, parity was shown to have an effect on milk yield in asses with reduced milk yield in donkeys with a parity of 3 vs 4 (Muhatai et al., 2017). Age and parity are often very closely related in these studies, making it difficult to differentially assess their effects. In other species, such as cattle, it has been widely demonstrated that milk yield during the first lactation is lower compared to that of subsequent pregnancies (Congleton Jr and Everett, 1980; Wood, 1980; Friggens et al., 1999). In addition, the effect of age was determined to be non-linear with an increase in milk production up to the ages of between 6 and 8 years of age and a subsequent decrease as cows grow older (Lush and Shrode, 1950; Lubritz et al., 1989).

Different methods have been used to estimate milk yield throughout the lactation period. The one which is considered to be the most precise involves the use of deuterated or tritiated water (Doreau and Boulot, 1989). It has not been used in more recent studies, however, probably due to the difficulties inherent in applying such methodology in horses. Milk yield has also been estimated through milking, but results are difficult to compare from one study to another as protocols vary widely (Doreau and Martuzzi, 2006). The main difference, between studies lies in whether oxytocin was used or not to induce milk ejection prior to milking. The third method is the weigh-suckle-weigh (WSW) method. WSW was successfully applied to mares in several studies (Gibbs et al., 1982; Trottier et al., 2006) and is mainly applied in nursing mare that are unaccustomed to being milked, unlike dairy mares. This method is considered reliable if measurements are repeated (Doreau and Martuzzi, 2006). Ways of estimating the quantity of milk ingested per day by the foal differ between studies. On the one hand, Gibbs et al. (1982), performed the WSW procedure four times during daytime and four times at night, at 3-h interval. Next, the quantities ingested by the foal were added up to estimate its daily intake. On the other hand, Trottier et al. (2006), performed the WSW procedure seven times at 1-h interval and used the average quantity of milk ingested during each bout to extrapolate quantity of milk ingested by the foal over a 24-h period. In order to compute the 24-h extrapolation, authors measured the duration and frequency of nursing bouts by observing natural

nursing. So far, no comparison between results obtained by milking or WSW is available (Doreau and Martuzzi, 2006). Thus, the main purpose of this study is to compare milking and WSW performed on the same animals over a period of two consecutive years. In addition, the influence of the mare's live weight, age, parity, year of lactation and foal gender was studied.

Material and methods

Animals

Sixty-five Anglo-Arabian type mares were involved in the study, 39 in 2018 and 26 in 2019, 51 were multiparous (26 in 2018 and 25 in 2019) and 14 were primiparous (13 in 2018 and 1 in 2019). Multiparous mean parity was 2.71 ± 0.88 (Supplementary Fig. S1). Mean age was 8.6 ± 2.6 years old (8.4 ± 2.9 in 2018 and 9.0 ± 2.1 in 2019) and mean weight after foaling was 561.46 ± 46.51 kg (570.67 ± 40.25 kg in 2018 and 547.30 ± 52.48 kg in 2019). One month after foaling, mean BCS was 3.3 ± 0.6 . This factor was not taken into account for further analysis as the population was quite homogenous regarding this measurement with no individuals presenting extreme values ($BCS < 1.5$ or $BCS > 4$). From 11 months of gestation to foaling, mares were fed daily with 8 kg of hay, 3 kg of haylage and 2 kg of pelleted concentrates (60% barley, 32.6% soy meal and 7.4% mineral and vitamin supplement) (Martin-Rosset, 2015). Precision concerning hay and haylage quantities and qualities are presented in Supplementary Table S1. Foalings took place each year between March 1st and May 15th and all were attended by farm staff. All foals were born healthy without human assistance, with 33 females (20 in 2018 and 13 in 2019) and 32 males (19 in 2018 and 13 in 2019). From foaling to 3 days postpartum, mares and foals were housed in 16 m square boxes with straw bedding. They had daily access to the paddock from 1000 h to 1600 h. During this time, mares were fed twice a day with increasing quantities of haylage and concentrates in order to increase energy intake following INRAE recommendations (Supplementary Table S1) (Martin-Rosset, 2015). From 4 days postpartum to weaning, mares and foals were kept in a group at pasture. During this period, neither concentrates nor dry forages were distributed to the mares, grazing being their unique feeding source. Pasture was managed as rotational grazing and mares and foals were moved from one pasture to another whenever grass height reached around 5 cm (every 2–5 days). Grass quality did not vary between the different pastures within the year although a difference in mean grass quality was identified between 2018 and 2019 in terms of the energy available in the feed (UFC: 0.66 ± 0.05 and 0.68 ± 0.04 , respectively) and of the quantity of digestible proteins (MADC: 102.52 ± 21.46 g per kg of DM and 76.57 ± 44.42 g per kg of DM, respectively). It is known, however, that contrary to ruminants, horses can compensate a lower quality of feed by increasing their feed intake (Hoskin and Gee, 2004). Thus forage was considered available *ad libitum*. Water was provided *ad libitum* at all times. Foals were weaned at 6 months.

Milk quantity estimation

Milk yield was evaluated by direct measurement (milking) and by a proxy measure (WSW). No oxytocin was used at any stage of the protocol. Five lactation times were considered: day 3, day 30, day 60, day 90 and day 180. At each step the same protocol was run. At 0800 h, mares and their foals were moved from their pasture to the experimentation location. Mares were weighed and then foals were muzzled from 0830 h to 0900 h. After 3 h the first milking (M1) was performed using a manual milker, on both teats. Thirty minutes after the first milking, a second milking (M2) was

performed. Thirty minutes after the second milking, the muzzle was taken off and the foal was weighed before being allowed to suckle. Suckling time was measured and, when suckling was ended by the foal, its weight was measured a second time. This procedure provided the first weigh-suckle-weigh (**WSW1**). Then, the foal was muzzled a second time for the second weigh-suckle-weigh (**WSW2**) which occurred 1 h after WSW1. The milk production of the mare is thus estimated by the difference in weight measured between the two weighings. The whole procedure is summarised in Fig. 1. Weighings from day 3 to day 180 of lactation for WSW measurements were performed using an automated scale (PUEC31, Radwag electronics, Poland). Milkings were performed using a manual milker. When foals defecated while suckling, the faeces were weighed and, when they urinated, it was noted and the measurement was not taken into account for further analysis. Measured suckling time referred to effective time spent suckling by the foal, the chronometer was stopped if the foal interrupted the bout and, started again when it returned to the udder.

Statistical analysis

Statistical analyses were performed using the R software (R. Core Team, 2021). Raw data are expressed in mean ± SD. Initially, quantities of milk obtained from M1 were assumed to correspond to the maximum capacity of the mares' udder, whereas those obtained from M2 reflected the capacity of the udder to refill in half an hour. For 16.9% of measurements, however, M2 was greater than M1. In addition, suckling times and differences of weight between the two weighings measured for WSW1 were influenced by the foal's 4-h fasting. Indeed, foals spent more time suckling during WSW1 than WSW2 and, after some time, no more swallowing was visible. This suckling appeared to provide comfort rather than feeding. Thus, M1 and WSW1 data were not taken into account for further statistical analyses.

The first step was to compare M2 and WSW2 data sets. After editing (discarding individuals with missing values or outlier values), 23 individuals were kept. Pertinence of WSW as a proxy measurement was assessed by computing the correlations between M2 and WSW2 at each timepoint. The second step focused on M2 measurements to study factors influencing milk yield. Analyses were performed on 57 individuals and two sets of variables were considered. The first was a subset of explanatory variables: the live weight of the mare 24 h after parturition (**LW**), parity, age, year

of lactation and foal gender (Table 1). The second was a response matrix containing milk yield data from milking at the five time-points of the lactation. Principal component analysis (**PCA**) was performed on the explanatory matrix. The relationship between milk production and environmental factors was studied using a redundancy analysis (**RDA**). PCA and corresponding graphs were performed using the ade4 (Thioulouse et al., 2018) and adegraphics packages (Siberchicot et al., 2017).

Redundancy analysis is an extension of multiple regression methods to model multivariate response data (Legendre and Legendre, 2012) by a set of explanatory variables. It allowed us to test the significance of variables by performing ANOVA-like permutation tests. Redundancy analysis computations were performed using the vegan package (Oksanen et al., 2020).

Three RDA models were computed with different factors studied:

- Model 1: mare age, mare parity, mare LW, foal gender and year,
- Model 2: mare age and LW,
- Model 3: mare parity and LW.

For each model, three consecutive ANOVA-like analyses were computed on RDA results. The first allowed to determine the significance of the model, the second to determine which axes were significant and the third to study the significance of the studied factors themselves.

Results

Milking and weigh-suckle-weigh

Second milking data measured the milk production of the mare within 30 min at different timepoints during the lactation period (day 3: 252.0 ± 77.3 g; day 30: 288.7 ± 135.8 g; day 60: 296.3 ± 84.3 g; day 90: 289.3 ± 133.1 g; day 180: 204.3 ± 80.2 g). Second weigh-suckle-weigh was used as a proxy method to measure milk production at different timepoints during the lactation period (day 3: 523.1 ± 282.9 g; day 30: 682.3 ± 295.3 g; day 60: 690.8 ± 279.1 g; day 90: 650.8 ± 339.1 g; day 180: 469.2 ± 339.14 g). Correlations between M2 and WSW2 were very low, the largest value was equal to 0.41, and between -0.05 and 0.15 for the other times of measurement (Table 2). Moreover, comparing individual curves, no clear pattern emerged from WSW2

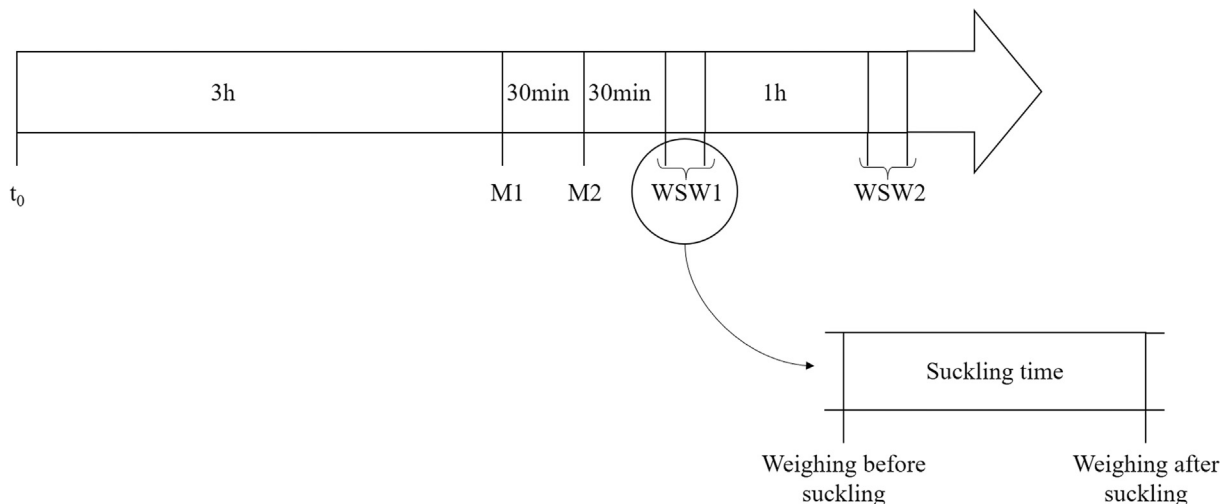


Fig. 1. Protocol realisation in the mare with t_0 the beginning, M1 the first milking, M2 the second milking, WSW1 the first weigh-suckle-weigh and WSW2 the second weigh-suckle-weigh.

Table 1

Distribution of explanatory variables for the individuals included in statistical analysis for 2018 and 2019.

Item	2018	2019	2018–2019
Number of individuals	34	23	57
Number of primiparous	11	1	12
Number of multiparous	23	22	45
Mean parity of multiparous	2.81 ± 0.83	2.58 ± 0.93	2.71 ± 0.88
Number of female foals	17	12	29
Number of male foals	17	11	28
Mean LW	570.51 ± 42.68	550.83 ± 50.57	562.57 ± 46.62
Mean age	8.41 ± 2.80	8.91 ± 2.04	8.61 ± 2.51
Mean foaling week	15.21 ± 1.70	15.83 ± 1.23	15.46 ± 1.55

Abbreviations: LW = live weight (kg) of the mare 24 h after foaling.

representative curves (Fig. 2A) whereas a stable trend appeared for M2 representative curves (Fig. 2B).

Principal component analysis

Principal component analysis was computed to study the relationships between the different milk quantities measured during lactation. A correlation circle was plotted in order to visualize the relationships between the different measurements. Explanatory variables (mare LW, mare parity, mare age, year of lactation and foal gender) were plotted on the correlation circle as supplementary variables (Fig. 3). The first two dimensions explained respectively 52% and 17% of the variability. The first dimension represented a general size effect while the second corresponds to a pattern effect. Thus, with a constant mean production indicated by the first dimension, an opposition between production at day 30 and at day 90 is observed. Moreover, mare age and LW were slightly positively correlated to the first dimension. In contrast, the year of lactation was negatively correlated with the first dimension. Studying the position of qualitative factors on the individual factor map, parity appeared correlated to the first dimension (Fig. 4). Interestingly, age was also linked to the first dimension but the relationship did not appear linear (Fig. 5) with ages '6' and '13' being offset.

Redundancy analyses

The three RDA models appeared significant ($P < 0.05$) and they explained respectively 21%, 17% and 13% of the total inertia. For each model, only the first axis was significant ($P < 0.05$) explaining respectively 85%, 93% and 90% of the model inertia. Regarding model 1, only mare LW and age tended to be significant ($P = 0.07$ and $P = 0.06$, respectively). Thus, model 2 was computed with mare LW and age only. Model 3 was computed due to the strong correlation between age and parity in our population. In model 3 mare LW and parity appeared significant ($P < 0.05$). The results of variances for the three models are presented in Table 3.

Table 2

Correlations between M2 and WSW2 at five times of the lactation in the mare.

Item	WSW2_3	WSW2_30	WSW2_60	WSW2_90	WSW2_180
M2_3	0.41				
M2_30		-0.02			
M2_60			0.05		
M2_90				-0.05	
M2_180					0.15

Abbreviations: M2 = second milking; WSW2 = second weight-suckle-weight procedure; M2/WSW2_3 = M2 or weight-suckle-weight (WSW) at day 3; M2/WSW2_30 = M2 or WSW at day 30; M2/WSW2_60 = M2 or WSW at day 60; M2/WSW2_90 = M2 or WSW at day 90; M2/WSW2_180 = M2 or WSW at day 180.

Discussion

Sampling method

The first hypothesis concerning milking in this study was that, after 3 h without being emptied, milking allowed to express the maximum capacity of the udder. We were not able to verify this hypothesis for all mares involved in the protocol. Milking having been performed without oxytocin injection, it is possible that the cisternal milk was not always fully sampled. Indeed, it is known that oxytocin is necessary for milk ejection and allows the collection of cisternal as well as alveolar milk (Ellendorff and Schams, 1988). An increase in oxytocin provoked by the first milking may have consequences on the second by increasing milk yield. Thus, the mare's response to stimulation during milking seems to be variable among individuals.

This study aimed to find an alternative to milking in order to estimate milk production in mares. Thus, a method of WSW was used. WSW was chosen because of its previous application in several experimentations on milk production in mares (Trottier et al., 2006; Santos and Silvestre, 2008; Centoducati et al., 2012). Moreover, it can be used on all individuals, even nursing mares not accustomed to being milked. When drawing individual curves using WSW2 data, however, no clear pattern was highlighted. It was also observed that, WSW2 data are not correlated to M2 data which corresponds to a direct measurement of mare milk yield. The main difference between previous studies using WSW and the present one is the measurement frequency. Indeed, foals were previously monitored over a 24-h period (Santos and Silvestre, 2008; Centoducati et al., 2012) and not over a single suckling bout. These observations led to doubt about the accuracy of WSW as a proxy for milk yield estimation when applied without repetition. No repetitions were performed during this experiment in order to determine if a simplified procedure could be used to monitor milk production in the mare. Indeed, even though performing repetitions over a 24-h period, or more, was demonstrated as accurate, the method cannot be applied in practice by mare milk producers needing to determine which mare is more suitable for milking. A slight correlation was found at day 3 even though WSW method was previously identified as less precise at the beginning of the lactation period than later (Doreau and Martuzzi, 2006). We observed that quantities measured using WSW2 method were higher than quantities obtained during M2. In a review, Doreau and Martuzzi (2006), had stated that milking without oxytocin might lead to an underestimation of milk yield.

Factors influencing milk yield

An effect of the mare live weight 24 h after parturition was observed with thinner mares producing less milk than heavier ones. The effect of the mare's live weight was previously mentioned (Gibbs et al., 1982; Oftedal et al., 1983), and even used as a means of estimating milk yield (Doreau, 1994). In these studies, however, it was the live weight during lactation that was taken

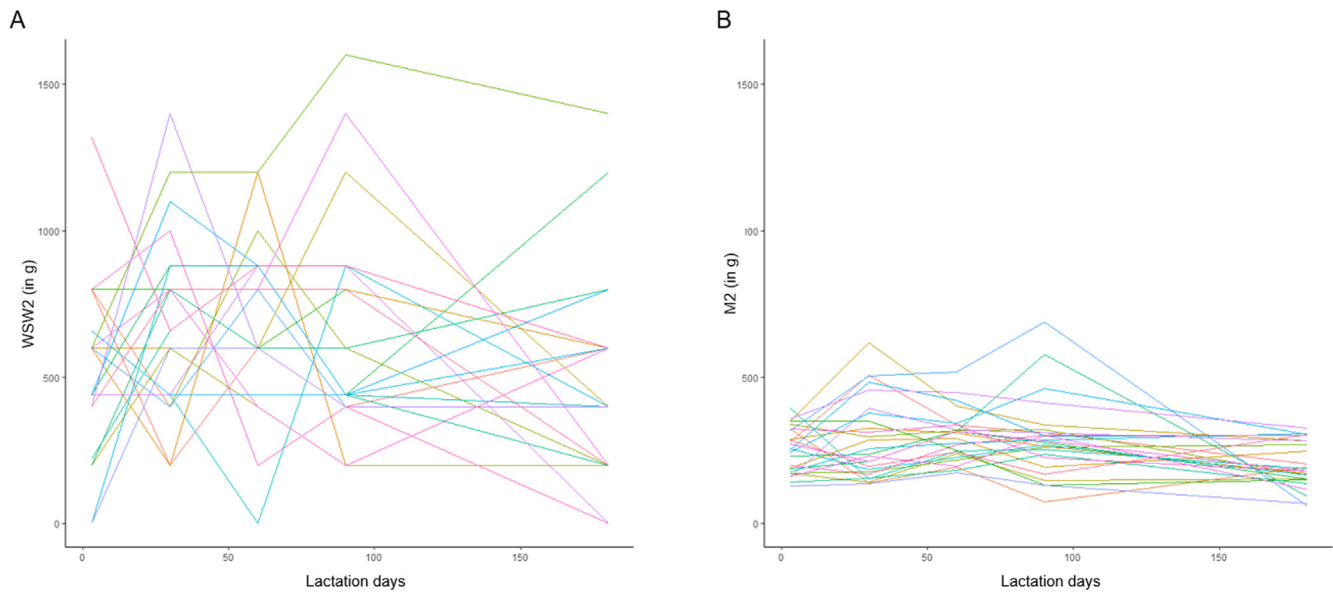


Fig. 2. Representative curves of individual mare milk production with measurements performed at 3, 30, 60, 90 and 180 days of lactation, A – Milk production being estimated through the second weight-suckle-weight (WSW2) data, B – Milk production being measured through the second milking (M2) data.

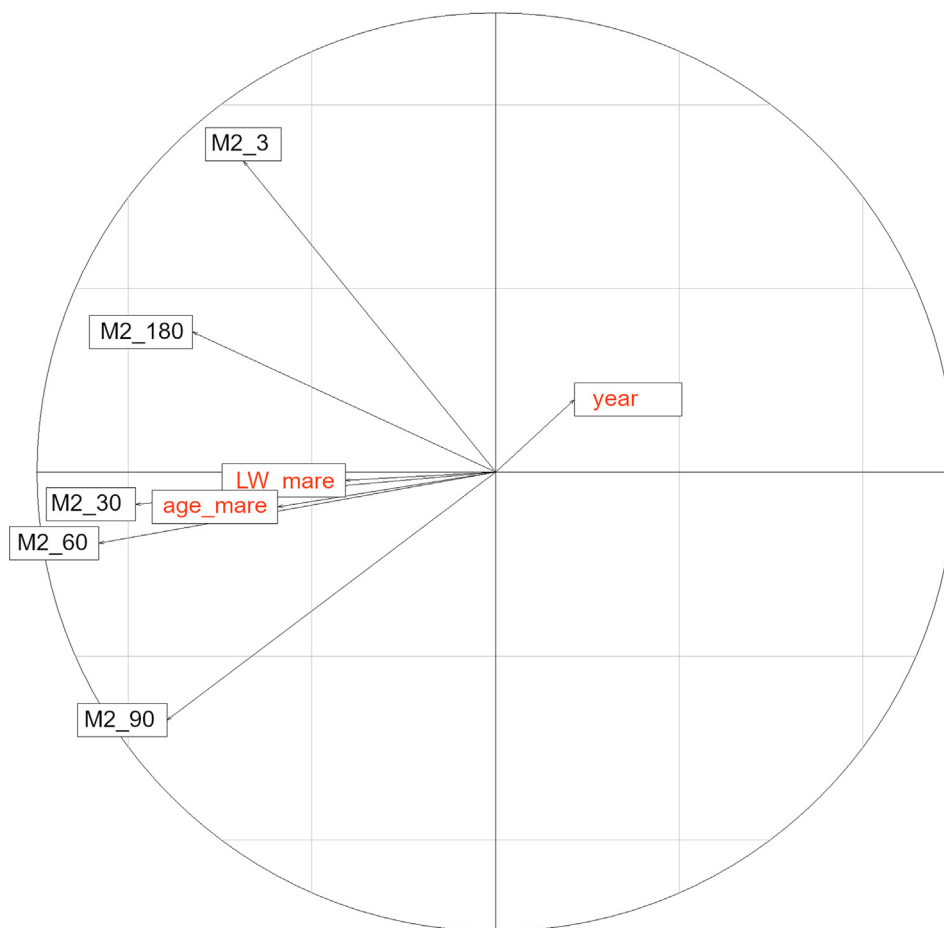


Fig. 3. Correlation circle representation of the active variables in the two first dimensions of principal component analysis (PCA). Supplementary variables are shown in red. With 'M2_3', 'M2_30', 'M2_60', 'M2_90' and 'M2_180' being respectively the quantity of milk measured by milking at 3, 30, 60, 90 and 180 days of lactation, respectively; 'LW mare' indicates the live weight (kg) of the mare 24 h after parturition; 'year' the year studied (2018 or 2019); 'age mare' mare's age during lactation.

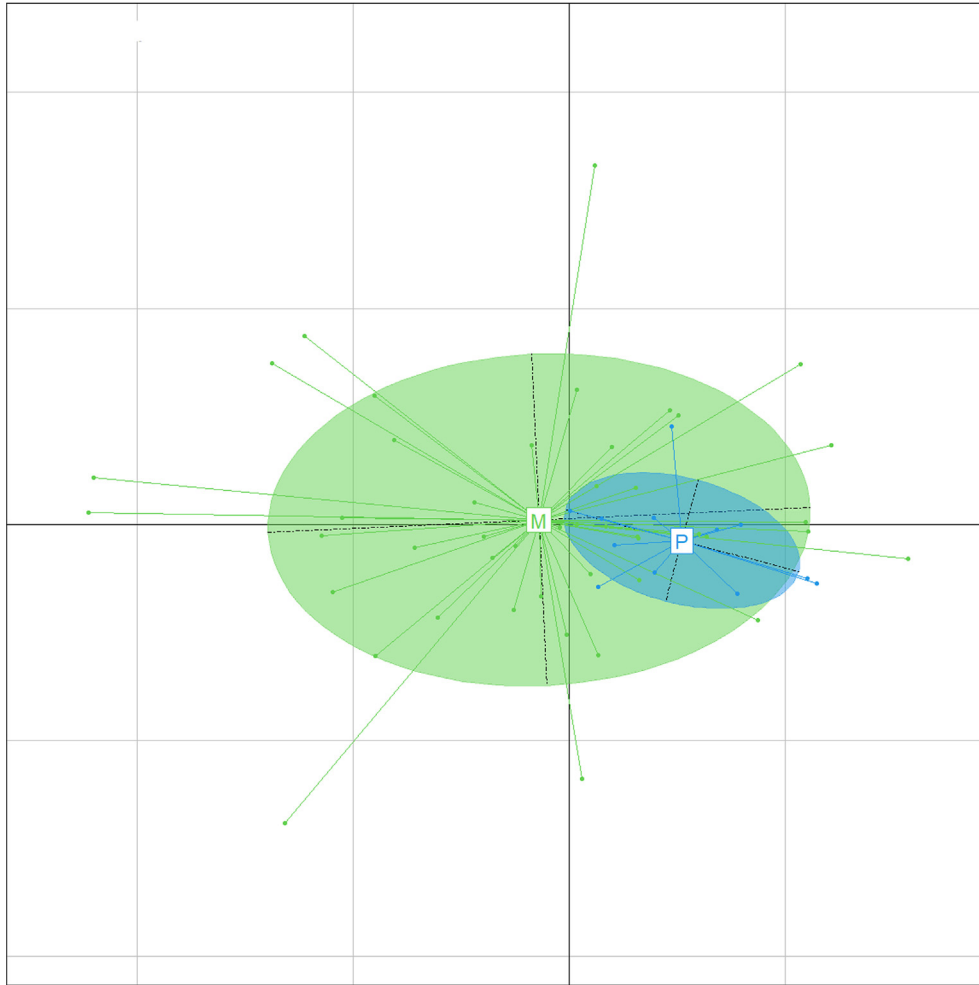


Fig. 4. First factorial map (axes 1 & 2). Blue and green points stand for primiparous (P) and multiparous (M) mares, respectively. The two different groups are labelled within the 95% inertia ellipse.

into account. In the current paper, we demonstrated the effect of the live weight at the beginning of lactation. This effect can be compared to what was previously shown by Doreau et al. (1993) concerning body condition before foaling. They demonstrated that mares with higher body conditions at foaling produced more milk. Body condition and weight being correlated (Martin-Rosset, 2015), our results are in accordance with theirs.

We identified an effect of age, with younger mares producing less milk than older mares. Maximum potential production has been cited to be reached at 7 years old for nursing mares (Doreau and Martuzzi, 2006). In the present study, the effect of age was highlighted but we were not able to identify an increase in milk yield until 7 years old followed by a drop in milk production for mares older than 7. Regarding the high inter-individual variation which would enable us to observe such a pattern, it would be necessary to monitor individual milk production over several years. Nevertheless, the relationship between age and milk yield appears non-linear which is in accordance with previous studies.

We demonstrated an effect of parity on milk yield with primiparous mares appearing to produce less milk than multiparous. This observation is in accordance with a previous study in which authors did find a significant effect (Gibbs et al., 1982), or a trend (Doreau et al., 1991), of parity with lower milk yield in primiparous. The latter, suggested an incomplete development of mammary gland tissues in primiparous. Indeed, an increase in

mammary size is observed from 5 days prepartum in the mare (Peaker et al., 1979). In cows, part of mammary development occurs also during pregnancy due to oestrogen and progesterone secretions (Tucker, 1987). At the end of the lactation period, a rapid decrease in mammary epithelial cells occurs but some remain if the dry period is not extended. A similar pattern might be observed in horses.

Limits

All measurements were performed in the afternoon between 1330 h and 1630 h although it has been demonstrated that jennies produce more milk in the morning than in the evening (D'Alessandro and Martemucci, 2012), +18.6% more at 0700 h than at 1900 h with 8-h interval between milkings. This was also shown in another study (Alabiso et al., 2009), with a 240 g reduction of milk quantity when milking occurred in the evening compared to the morning. It is possible that a similar difference between the time of the day also exists in mares. Indeed, foals suckle more during daytime (Duncan et al., 1984; Trottier et al., 2006) thus, reduced secretion during the night may represent an adaptation of the mare to the foal's needs. We might also suspect, at least for the milking at day 3, an effect of habituation. Indeed, unlike primiparous, the majority of multiparous mares (44 out of 51) had been milked before. It has been demonstrated that mare unac-

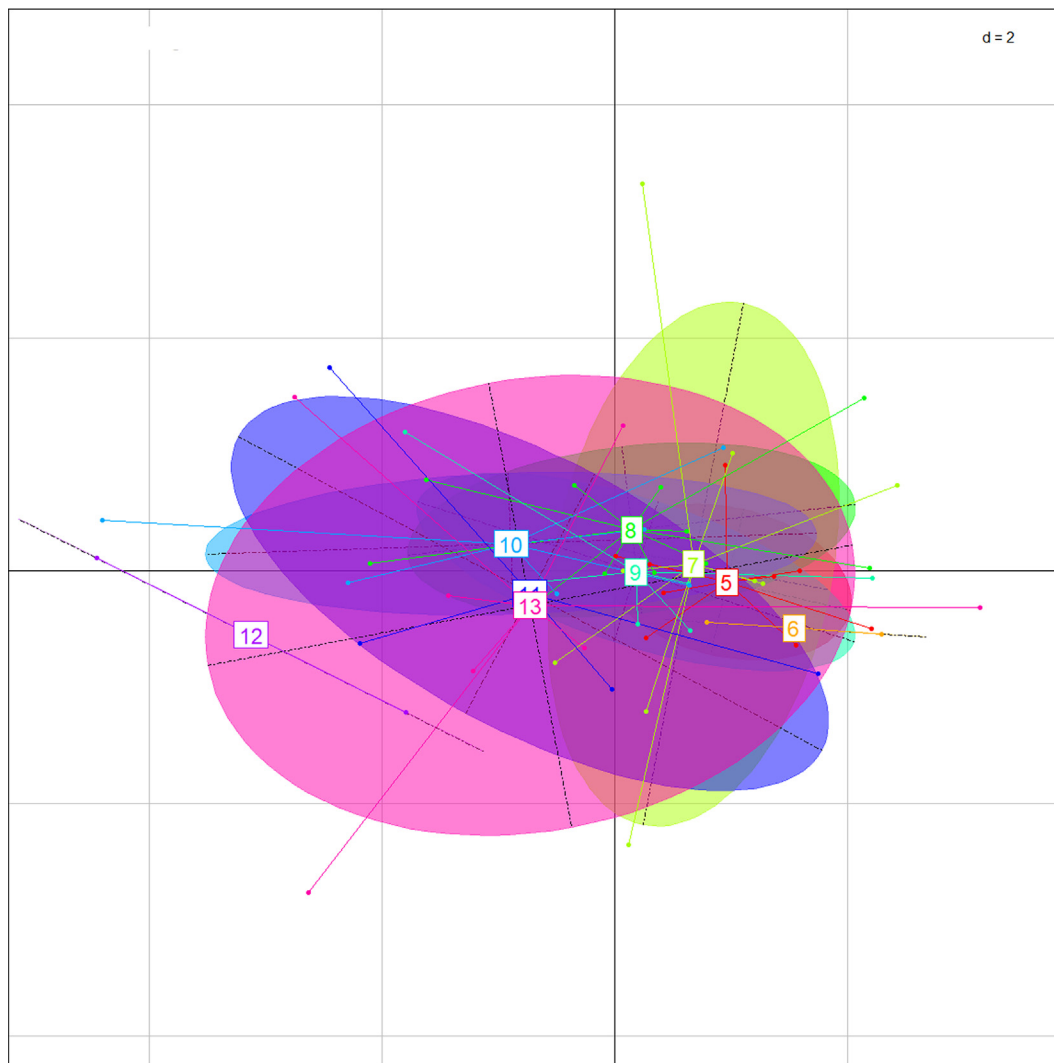


Fig. 5. First factorial map (axes 1 & 2). Red, yellow, light green, green, light blue, blue, dark blue, purple and pink points stand for mares aged 5, 6, 7, 8, 9, 10, 11, 12 and 13, respectively. The different groups are labelled within the 95% inertia ellipse.

Table 3

Results of variance analyses for three RDA (redundancy analysis) models computed to study the effect of different factors on milk yield. The column 'variance' indicates the percentage of total variance explained by each studied variable.

Item	Model 1		Model 2		Model 3	
	Variance	P-value	Variance	P-value	Variance	P-value
Parity	6%	0.48			31%	<0.05
Foal sex	5%	0.64				
Year	13%	0.14				
Mare LW	18%	0.07	24%	<0.05	36%	<0.05
Mare age	18%	0.06	47%	<0.05		

Abbreviations: LW = live weight (kg) 24 h after foaling.

customed to being milked tend to produce less milk (Bellomo et al., 2005; De Palo et al., 2018), probably due to stress as cortisol tends to decrease milk secretion.

Conclusion

The first aim of this study was to evaluate the use of WSW performed without repeated measurements at different timepoints during lactation, in order to estimate milk yield. A correlation

between this estimate and a direct milk measurement could not be demonstrated. It seems to indicate that the WSW method cannot be used for milk yield estimation in the mare without repeating the measurement at each timepoint. The second aim was to identify factors influencing milk yield. This step focused on results from direct measurements of milk quantities produced by the mares. The effects LW, age and parity of the mare were identified. Because of the partial confusion between age and parity, it was not possible in the study population to clearly identify which effect,

parity or age, had the greatest impact. A study with young and old primiparous animals and young and old multiparous mares would be needed to clarify this point.

Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.animal.2022.100498>.

Ethics approval

The protocol was approved by the local animal care and use committee ("Comit  des Utilisateurs de la Station Exp rimentale de Chamberet") and by the regional ethical committee ("Comit  R gional d'Ethique pour l'Exp rimentation Animale du Limousin", approved under N  C2EA - 33 in the National Registry of French Ethical Committees for animal experimentation) under protocol number APAFIS#14963-2018050316037888 v2. All experiments were performed in accordance with the European Union Directive 2010/63EU. The study took place in the equine research station registered by the French ministry of research under research agreement C1903602 valid until March 22, 2023.

Data and model availability

Data were deposited in an official repository (<https://doi.org/10.15454/3LPGKP>). The model was not deposited in an official repository, however, it is available upon request.

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Declaration of interest

None.

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