UNIVERSITÀ DEGLI STUDI DI PADOVA

Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente

Corso di laurea magistrale in Scienze e Tecnologie Animali

TESI DI LAUREA

Suckling of dairy calves by their dams: consequences on animal performances, behaviour and welfare

Relatore
Prof. Enrico Sturaro
Correlatori
Dott. Bruno Martin
Dott. Dominique Pomiès

Laureanda
Alessandra Nicolao
Matricola n. 1148571

ANNO ACCADEMICO 2017/2018

ABSTRACT

In most European dairy farms, calves are separated from their mothers immediately or within few hours after birth (Le Cozler et al., 2012). This separation of the calves from mothers allows to control calves' colostrum and milk intake without affecting the quantity of milk produced by cows. During the first 1-2 weeks of life, calves are housed in individual pens in order to have a better supervision on milk ingestion and health control. After this time female calves, which are the future replacement heifers, are reared in multiple pens and fed with milk replacers or non-marketable milk. Subsequently they begin to receive gradually little quantities of solid food until the time of weaning. Male calves, instead, are sold during the first 14 days for fattening in specialized farm.

The early separation from calves and mothers is increasingly questioning the society about animal welfare and consequently requires farmers to be engaged with controversial issues related to animal care. In particular Ventura et al. (2013) evidenced that the early separation of calf and mother is perceived as a problem specifically by citizens who are far from livestock production. Some scientists also claimed that "we need to bring back dairy calves to their mother" (Agenäs, 2017). But the consequences of this practice have been studied mainly from the animal welfare point of view and not from the animal performances point of view (Veissier, Caré, & Pomiès, 2013). As regards the performance of cows (production, reproduction, health of the udder and milk quality) and calves (growth, health), they were studied in particular in the context of once daily milking, where significant losses in milk production are observed (D Pomiès et al., 2010).

The aim of this project was to study the impact of a suckling rearing system on milk yield, milk composition, growth of calves and the animal behaviour and welfare.

The project was carried out between February and July 2018 at INRA, UE Herbipole, in the site of Marcenat, France. The experiment involved 28 cows with their calves that were monitored for 13 weeks after calving. Parturitions took place between the end of February and the end of April. The 14 Montbéliarde (Mo) and 14 Holstein (Ho) cows involved in this experiment were selected on the basis of their lactation rank, date of calving, milk index and sex ratio of calves. Two experimental groups (group 'Control' and group 'Mother'), composed of 7 Mo and 7 Ho cows each were made so that within each breed, the lactation rang, the date of calving and the milk yield (index for primiparous cows and total milk yield during the previous lactation for multiparous cows) were equivalent.

In the *'Control'* group, the calves were separated from their mothers within the few hours after birth and were housed in individual pens, fed twice a day with milk from the tank for 7 days. Successively they were placed in a collective park for 9 weeks and fed with bulk milk (up to 10 kg/day) and concentrate (up to 2 kg/day) by an automatic feeder and quality hay fed *ad libitum*. Weaning was made after 10 weeks when calves' live weight was about 100 kg. Then, calves were fed with concentrate (up to 4 kg/day) and good quality hay fed *ad libitum*.

In the *'Mother'* group, the calves spent 5 days after parturition in an individual calving pen with their mother in order to allow mother-calf attachment. Cows were taken to the parlour to be milked twice daily at approximately 07:30 and 16:30. After this period, both cow and calf were moved to the collective park for 9 weeks. Cow-calf contact was allowed during the day: calves had free access to the stabling of mothers for suckling between the return of the morning milking and the departure for the evening milking. During the night they were separated from the mothers and housed in the calves' collective park, next to the stabling of the mothers. They could see their mother, at least at the level of the separation of the 2 parks. Calves suckled the cows freely during the day and had access to concentrates and good quality hay *at libitum* 24h a day. Weaning was made after 10 weeks when the calves' live weight was about 100 kg. Then the calves were fed with concentrate (up to 4 kg/day) and good quality hay *ad libitum*.

During the experiment, measurements and analyses concern milk production and composition, cow's body weight (BW) and Body Condition Score (BCS), calves' growth, individual quantity of milk and concentrates ingested by calves in the group 'Control', quantity of concentrate ingested by calves group 'Mother', health events of cows and calves and different types of behavioral observations on all the animals involved in the study.

Our results showed that from the point of view of production performances, this system has a strong impact on milk production in terms of quantity and composition. Loss of milk is substantial compared to a classic rearing system. The reduction in milk fat content and, in our case, the increase in milk protein content affects the composition and quality of the milk. On the other hand, the performances of the calves were positively affected by this practice.

From the behavioural point of view, this system has influenced the maternal behaviour of the cows in terms of preferences towards their calves and has influenced the social interactions of the calves 'Mother': the presence of cows stimulates the social interaction of young animals. The more critical aspect of this practice was the moment of weaning. It is important to remember that weaning is always a stressful moment for calves, even in the classic breeding system (Weary & Chua, 2000).

But in suckling rearing system, the impact on animal welfare was even strongest, and above all it involves both calves and cows. The separation of the animals caused a strong stress that lasts for a few days and affects the production of milk for the cows and the growth of the calves.

In conclusion we can say that the natural suckling system affects both the production performance and the behaviour and welfare of the animals. The presence of positive and at the same time negative aspects does not allow us to give a unidirectional opinion on the evaluation of this system, but further studies are required to evaluate this practice more completely, especially from the economic point of view.

RIASSUNTO

Nella maggior parte delle aziende lattiero-casearie europee, i vitelli vengono separati dalla madre immediatamente o entro poche ore dalla nascita (Le Cozler et al., 2012). La separazione dei vitelli dalle madri consente di controllare l'assunzione di colostro e di latte dei vitelli senza influire sulla quantità di latte prodotto dalle vacche. Durante le prime 1-2 settimane di vita, i vitelli vengono stabulati in recinti individuali per avere una migliore supervisione dell'ingestione di latte e del controllo dello stato sanitario. Dopo questo periodo le femmine, in quanto future giovenche da rimonta, vengono trasferite in box multipli e alimentate con sostituti del latte o con latte non commerciabile. Successivamente iniziano a ricevere gradualmente piccole quantità di alimento solido fino al momento dello svezzamento. I vitelli maschi, invece, vengono venduti durante i primi 14 giorni di vita alle aziende specializzate per l'ingrasso.

La società si interroga sempre più spesso sulla separazione precoce dei vitelli dalle madri, in particolare per quanto riguarda il benessere degli animali. Questo comporta che gli agricoltori siano sempre più spesso coinvolti in controverse questioni relative alla gestione degli animali. In particolare, Ventura et al. (2013) hanno evidenziato che la separazione precoce è percepita come un problema soprattutto dai cittadini che non sono a diretto contatto con il settore. Alcuni scienziati ugualmente hanno affermato che "dobbiamo riportare i vitelli da latte alla loro madre" (Agenäs, 2017). Ma le conseguenze di questa pratica sono state studiate principalmente dal punto di vista del benessere degli animali e poco dal punto di vista delle performance produttive (Veissier, Caré, & Pomiès, 2013). Per quanto riguarda le performance delle vacche (produzione, riproduzione, salute delle mammelle e della qualità del latte) e dei vitelli (crescita, salute), sono state studiate solo nel contesto di una sola mungitura giornaliera, in cui sono state osservate perdite significative nella produzione di latte (D Pomiès et al., 2010).

Lo scopo di questo progetto è stato quello di studiare l'impatto di un sistema di allevamento ad allattamento naturale sulla produzione e sulla composizione del latte, sulla crescita dei vitelli e sul comportamento e il benessere degli animali.

Il progetto è stato realizzato tra Febbraio e Luglio 2018 all'INRA, UE Herbipole, nel sito di Marcenat, Francia. La prova ha coinvolto 28 vacche con i rispettivi vitelli i quali sono stati monitorati per le 13 settimane successive al parto. I parti hanno avuto luogo tra la fine di Febbraio e la fine di Aprile. Le 14 vacche Montbéliarde (Mo) e le 14 vacche Holstein (Ho) coinvolte in questo studio sono state selezionate sulla base del loro periodo di lattazione, della data del parto, del loro 'indice latte' e del

sesso del loro vitello. Sono stati studiati due gruppi di animali (gruppo 'Control' e gruppo 'Mother'), composti rispettivamente da 7 vacche Mo e 7 vacche Ho ciascuno. I due gruppi sono stati formati equivalentemente in termini di razza, periodo di lattazione, data del parto e produzione di latte (cioè, 'indice latte' per le vacche primipare e produzione totale di latte dell'ultima lattazione per le vacche pluripare).

Nel gruppo *'Control',* i vitelli venivano separati dalla madre entro poche ore dalla nascita e stabulati in box individuali per 7 giorni dove venivano alimentati due volte al giorno con il latte dell'azienda, distribuito in un secchio individuale provvisto di tettarella. Successivamente venivano trasferiti nel parco collettivo per 9 settimane e alimentati con latte sfuso (fino a 10 kg/d), concentrati (fino a 2 kg/d) somministrati da due distributori automatici e fieno *ad libitum*. Lo svezzamento è stato fatto dopo 10 settimane quando il peso vivo dei vitelli raggiungeva i 100 kg. L'alimentazione dopo lo svezzamento ha previsto concentrati (fino a 4 kg/d) e fieno di alta qualità *ad libitum.*

Nel gruppo *'Mother'*, i vitelli trascorrevano i primi 5 giorni di vita in un box parto individuale con la propria madre, allo scopo di permettere la formazione del legame madre-vitello. Le vacche venivano munte due volte al giorno tutti i giorni verso le 7:30 e le 16:30. Alla fine dei 5 giorni, ogni coppia vacca-vitello veniva trasferita nel parco collettivo per 9 settimane. Il contatto vacca-vitello veniva permesso durante la giornata: i vitelli avevano libero accesso allo stabilimento delle madri nel periodo che intercorreva tra il ritorno dalla mungitura mattutina e la partenza per la mungitura serale. Durante la notte, infatti, erano separati dalle madri e stabulati in un parco apposito per i vitelli che si trovava accanto allo stabilimento delle madri. In questo modo potevano vedere le loro madri almeno a livello della separazione dei due parchi. I vitelli potevano succhiare le vacche liberamente durante il giorno e avevano accesso a concentrati e fieno di alta qualità *ad libitum* 24 ore al giorno. Lo svezzamento è stato fatto dopo 10 settimane quando il peso vivo dei vitelli raggiungeva i 100 kg. L'alimentazione dopo lo svezzamento ha previsto concentrati (fino a 4 kg/d) e fieno di alta qualità *ad libitum.*

Durante la prova sono state effettuate misurazioni e analisi riguardanti la produzione e la composizione del latte, il peso vivo (BW) e il Body Condition Score (BCS) delle vacche, la crescita dei vitelli, la quantità di latte e di concentrati ingerita individualmente dai vitelli del gruppo 'Control', la quantità di concentrati ingerita dai vitelli del gruppo 'Mother', gli eventi sanitari di tutti gli animali e differenti tipi di osservazioni comportamentali.

I nostri risultati hanno mostrato che dal punto di vista delle performance produttive questo tipo di

sistema ha un forte impatto sulla produzione di latte in termini sia di quantità che di qualità. La perdita di latte è sostanziale se comparata al sistema classico di allevamento/produzione. La riduzione del contenuto di grasso, e nel nostro caso, l'aumento del contenuto proteico ha influito sulla composizione e sulla qualità del latte. D'altra parte, le performance dei vitelli sono state positivamente influenzate da questo sistema.

Dal punto di vista comportamentale, questo sistema ha influenzato il comportamento materno delle vacche in termini di preferenza nei confronti del proprio vitello e ha influenzato le interazioni sociali dei vitelli del gruppo 'Mother': la presenza delle vacche stimola l'interazione sociale dei giovani animali. L'aspetto più critico di questa pratica è stato il momento dello svezzamento. È importante ricordare che lo svezzamento è un momento di forte stress per i vitelli anche nei sistemi classici di allevamento (Weary & Chua, 2000). Ma nel sistema ad allattamento naturale l'impatto sul benessere degli animali è stato comunque più forte e soprattutto ha coinvolto sia i vitelli che le vacche. La separazione degli animali infatti ha causato un forte stress che è durato per alcuni giorni e ha influito sulla produzione di latte delle vacche e sulla crescita dei vitelli.

In conclusione, possiamo dire che il sistema di allattamento naturale influisce sia sulle performance produttive che su comportamento e il benessere degli animali. La presenza di aspetti sia positivi che negativi non ci permette di dare un'opinione unidirezionale sulla valutazione di questo sistema. Ulteriori studi sono dunque necessari per valutare questa pratica in modo più approfondito, in particolare dal punto di vista dell'impatto economico.

1. INTRODUCTION

1.1 Rearing dairy calves: current practices and societal implications

1.1.1 Classic rearing systems

In most European dairy farms, calves are separated from their mothers immediately or within few hours after birth (Le Cozler et al., 2012). During the first 1-2 weeks of life, calves are housed in individual pens. European regulations limits the confinement of the calves in the individual case to 8 weeks of age (Council of the European Union, 2009). This separation of the calves from mothers allows controlling calves' colostrum and milk intake without affecting the quantity of milk produced by cows. This strategy also hastens a return to reproductive cycling of mothers (Newberry 2008) and enables farmers to have a better supervision on health of calves.

After this time when calves stay in individual pens, female calves, which are the future replacement heifers, are reared in multiple pens and fed with milk replacers or non-marketable milk. Milk is distributed by an automatic milk feeder or by a collective trough/individual bucket. Subsequently the calves begin to receive gradually little quantities of solid food until the time of weaning. Male calves, instead, are sold during the first 14 days for fattening in specialized farms.

The weaning, that corresponds to the suppression of milk in the diet, generally occurs at 8-10 weeks of life (von Keyserlingk & Weary, 2007). Therefore, the alimentation is based on solid feed, such as concentrates and hay, in increasing amounts starting from 2 kg per day.

1.1.2 Current practices of suckling system

Early separation of the calf from the cow is a keystone of the modern dairy farming. Nevertheless there is a renewed interest in cow-calf rearing systems in dairy production (von Keyserlingk & Weary, 2007) because of improved cow and calf health and production (Fröberg et al., 2007) and because of the growing interest in ecological or organic milk, in which calves are sometimes kept with their dams for a period of time.

In countries where cattle breeds are not selected for very high milk production (mainly developing countries), natural suckling is a common practice. The presence of the calf under the mother activates the milk ejection mechanisms, the cow is milked and the calf can then suckle again freely (Krohn, 2001). This is also found in the Salers cow, where the presence of the calf is necessary for the cow to give her milk (Agabriel et al., 2014).

In organic livestock production the directives (directive (EG) Nr. 834/2007 and directive (EG) Nr. 834/2008) (The Couincil of the European Union & of the, 2007)(Council of the European Union, 2009) indicate to feed the calves with whole milk preferentially from their mother for a period of three months. However, calves are often separated from their mothers shortly after birth as in traditional livestock.

Some organic farmers are not satisfied with this situation, so they have introduced suckling systems. Their aim is to increase welfare, improve animal health and technical performances such as faster growth and higher weaning weight resulting in lower age or higher weight at first calving (Wagenaar, 2009). It is important to say that there is no a golden formula for how to apply a suckling system, every farm has to develop its own system. Wagenaar et al. (2009) studied the different suckling system practices in the Netherlands and they found that single suckling of the mother is rarely used but system with nurse cow is the most practiced. Anyway, the critical point always remains the weaning, because suckling creates a bond between the mother (or the nurse) and calf, so even a two-step weaning does not completely solves stress around weaning.

But Grøndahl et al. (2007) observed that the possibility to watch the natural suckling and communication between cow and calf, as well as the play behaviour of the calves increased the farmer's welfare at work, and, to their opinion, these benefits outweighed the stress response observed at separation, which was mainly vocalisation.

1.1.3 Criticisms from the society

The conception of animal welfare is gaining more and more importance in current social debates and the public's concern about the practices observed in rearing systems tends to increase strongly. For example, a study conducted in the United States and Germany (Busch et al., 2017) showed that the three main animal welfare concerns of society were movement restriction, painful procedures and the expression of natural behaviour.

In the dairy sector, the main concern in animal welfare is the mother-calf separation. The early separation from calves and mothers is increasingly questioning the society and consequently requires farmers to be engaged with controversial issues related to animal care. Ventura et al. (2013) evidenced that the early separation of calf and mother is perceived as a problem specifically by citizens who are far from livestock production. Some scientists also claimed that "We need to bring back dairy calves to their mother" (Agenäs, 2017).

Many farmers, on the contrary, claim that the separation after birth is the best choice and the less stressful strategy because the time necessary to create a bond between cow and calf is not given (Flower & Weary, 2001). This also allows them to monitor the colostrum intake of the calf in the first hours of life in order to ensure that the colostrum intake is sufficient for the development of its immune system. In addition, for economic reasons, they prefer to maximize milk for marketing and give non-marketable milk or milk powder to calves. For them, the early separation of cow and calf also reduces the risk of transmission of diseases between the cow and its calf because the duration of the contact is very limited (Busch et al., 2017).

Nevertheless the creation of the cow-calf bond has been found to occur very early, during the few hour after parturition (Edwards & Broom, 1982). Hudson & Mullord (1977) showed that 5 min of cow-calf contact is sufficient to create a maternal behaviour; in fact, if the calf is removed and reintroduced 12 hours later, the mother can recognize it. After 24h, even if the cow still shows signs of separation distress, the ability of the mother to recognize its calf is gradually decreasing. Avoiding a contact between mother and offspring in the early post-partum hours reduces the formation of a bond in 50% of the animals (Hudson & Mullord, 1977). In any case, the separation within 24h after birth causes a lower level of stress than separation after a few days. But from the health point of view, calves who spend some days with their mother show fewer diarrhoea's problems. This may be due to a higher ingestion of colostrum and consequently to the intake of higher levels of immunoglobulins (Weary & Chua, 2000). Other researches have shown that nursing can provide health and welfare benefits (Flower & Weary, 2001; Krohn, 2001).

Another criticism from the public is that the deprivation of natural suckling causes the expression of abnormal behaviour in calves, such as cross-suckling. In fact, the milk supply is often given in a bucket, teat-bucket or automatic feeder which does not always satisfy the calves' motivation to suckle (Krohn, 2001). In addition, the restriction of the quantity of milk used to stimulate solid feed ingestion, also contributes to the manifestation of abnormal behaviour, a sign of discomfort and distress.

Scientific studies related to the analysis of the opinions of people closely connected to the dairy industry or not showed that subjects who do not know about rearing practices tend to be opposed to early separation while farmers tend to be favourable. The farmers opinion is based on the knowledge of the general context of the farming system, while people not connected to dairy have more conflicting opinions because they find more difficulties to see the complexity of the farming

systems (Ventura et al., 2013). These different perceptions on animal welfare shows that there is no unified "public opinion" on animal welfare topics (Busch et al., 2017). Despite this, the common point found was that both people raise similar concerns about the calf's wellbeing such as emotional distress and health (Ventura et al., 2013) and that farmers would be willing to change their practices if a reasonable compromise was found.

1.2. Effects of suckling systems on cows and calves

1.2.1 Establishment of maternal behaviour and cow-calf bond

In the ungulates, the first hours after birth are essential for the creation of a bond between mother and child. In fact, there is a critical period in which if this relationship is not created the child will be rejected by his mother (Edwards & Broom, 1982).

At birth, it is necessary that cow begins to lick the veal. The behaviour of licking has numerous vital functions: it allows to remove the foetal membranes, to dry the calf and reduce the dispersion of body heat and to stimulate the new-born's activities such as breathing, circulation, defecation and urination. It also allows the creation of maternal filial bond thanks to the olfactory imprinting. Maternal behaviour develops with oxytocin, an hormone released a few days before calving. It promotes the identification of the calf by stimulating its individual recognition and memorization, in particular by the smell. The lack of licking behaviour increases the probability of reject by the cow towards its calf. Under natural condition, the salvation of the new-born depends totally on this. Lidfors et al., (1996) found that calves immediately separated from the mother after birth had a lower amount of antibodies in their blood than calves licked from their dam.

The social bond between a mother and her newborn is described as a preferential mutual, affectionate, emotional attachment that is relatively long lasting and survives temporary separations (Newberry & Swanson, 2008). This bond is characterized by affiliative behaviour such as allogrooming, provision of nourishment, warmth and protection, resting in contact, synchronizing activities, and maintaining proximity. Bonded individuals exhibit reinstatement behaviour when motivated to reunite after a period of separation, and greeting behaviour upon reunion (Newberry & Swanson, 2008). Often dams with their calves synchronize their activities more than unrelated animals in the same group (Veissier, Lamy, & Le Neindre, 1990).

Maternal deprivation influences the future development of calf social behaviour: it affects the manifestation of stereotypies and anxiety-induced behaviour (Wagner et al., 2015). Flower and

Weary (2001) showed that at the first approach with another similar, calves kept with their mother manifest more social behaviour than calves separated from the mother 24h after birth. Early contact with the dam can also affect the calves' later maternal behaviour: cows mother-reared spend more time to nursing and licking their own calf than artificially reared cows (Le Neindre, 1989).

Johnsen et al. (2015) have found that cow-calf attachment is not exclusively due to suckling; in fact, allowing the reunion of calves with their mothers during the night, they observed that calves fed only with automatic feeder have a latency of reunion with their mother shorter than calves that are nursed by their mothers. The preference for own cow/calves in term of time spent on allogrooming, stays in proximity and reunion after a period of separation, shows that a maternal bond develops and persists despite how much calves are nursed. These results could stimulate the research for the development of management and housing systems that allow cow-calves contact even in the absence of nursing.

1.2.2 Impact of natural suckling on physiological and physical state of calves and cows

Research has proven that nursing can provide health and welfare benefits for both calves and cows (Krohn, 2001).

1.2.2.1 Calves growth, health and behaviour

Some authors reported that rearing calves with their mother allows a faster growth, in particular when artificially reared calves are fed with a restricted milk diet (Flower & Weary, 2001). A higher weight gain at two months of age was also shown when the suckling period is limited at 10 days (Metz, 1987). The faster growth is first due to a higher milk consumption but Krohn et al. (2001) concluded that social interaction between cow and calf during the colostrum period could give a positive effect on the daily gain of the calf. Indeed, Lupoli et al. (2001) have shown an increase in oxytocin release in calves in response to both sucking and bucket drinking and they demonstrated that this hormone influences anabolic processes and growth. In addition, the highest levels of oxytocin cause a decrease in the level of cortisol which determines an anti-stress effect. It has also been found that suckling calves had a higher level of growth-promoting hormones than calves that were fed milk from buckets (Lupoli et al., 2000). Weary et al. (2000) did not find differences in calf gains but they reported that calves which spent more time with the cow before separation tended to manifest fewer events of diarrhoea during the first 3 weeks of life. This is probably due to the

high consumption of colostrum that allows to acquire higher immunoglobulin concentrations than calves separated early.

The most important behaviour in the reproductive process of mammals is suckling because it allows the transfer of milk from mother to the young. The deprivation of this natural behaviour frequently causes the manifestation of abnormal oral behaviours in artificial rearing calves that, on the contrary, are rarely observed in naturally suckled calves (B. A. Roth, Barth, Gygax, & Hillmann, 2009). During natural sucking, several physiological processes are activated, like the release of higher level of oxytocin which has "anti-stress" effects such as lower cortisol levels and decrease of blood pression (Lupoli et al., 2001). The most common abnormal oral behaviour on artificial reared calves is the cross-suckling. Roth et al. (2009) showed that calves allowed to meet their mother only for two times 15 min per day did not manifest cross-suckling unlike all artificial reared calves which do. Fröberg & Lidfors (2009) showed that artificially fed calves also develop cross-sucking behaviours when the suction motivation is continually stimulated by small amounts of milk equally distributed during the day. This demonstrates that milk intake via an artificial teat does not fully satisfy the calves' motivation to perform sucking behaviour. Tongue rolling is also an abnormal behaviour observed in animals fed with automatic milk dispenser but not observed in suckling ones (Sofie Fröberg & Lidfors, 2009). It has been observed that the dam influence positively the development of calves' species-specific behaviours, Veissier et al. (2013) hypothesized that cows help calves to focus their oral activities toward nutritive elements, such as milk or solid feed, or towards their dam, such as suckling or licking.

Suckling systems are more beneficial to the welfare of calves than the artificial rearing systems. In a free suckling system calves can be nursed by their mother, learn to eat roughage earlier and have more social contacts with other cows and calves than in artificial rearing systems (Krohn, 2001). At birth, calves reared with their dams stand earlier and are more reactive because the cow stimulates his calf just after calving (Johnsen, de Passille, et al., 2015). Studies based on calf activity in the first weeks of life show that calves with contact with mothers spend more time lying down than artificially fed calves, this may be due to the anti-stress effect of oxytocin (Sofie Fröberg & Lidfors, 2009). In terms of relationships with other similar, calves with contact with the mother for 2-12 weeks showed higher social activity than those separated from the mother within the first 24 h (Wagner et al., 2015). Similarly, Flower at al. (2001) showed that calves reared with their mother for 2 weeks manifested more intense social behaviour toward an unfamiliar calf than did those calves

separated from cow after 1 day of age. Long-term positive effects of staying longer with the cow can be seen when heifers are introduced into the dairy herd (Johnsen, de Passille, et al., 2015). Wagner et al. (2015) confirmed that rearing with a permanent access to the mother and the herd increases sociality leading to higher behavioural activity during isolation and affects physiological stress reactions. It seems quite clear from previous studies that the cow activates the calf, but there is little information about the effects of the presence of the calf on the cows' behaviour (Lidfors, 1996).

1.2.2.2 Cows

Positive effects on the cow behaviour, health and physiology from nursing the calf has not been much investigated. The few studies that have been carried out have found that dairy cows nursing their calf get a better udder health: suckling could decrease the risk of mastitis during the suckling period and in some cases even for some time after the end of suckling (Efsa et al., 2009; Krohn, 2001).

It is also been shown that suckling can influence the calving to conception interval. Metz et al. (1987) found that cows reared with their calves for 10 days after birth expressed a calving-conception interval significantly shorter than cows separated within 24 h from their calves. They also suggested that suckling could have positive effects on the recovery of the cow after calving, because it accelerates the involution of the uterus. Furthermore, they found that the duration of suckling period could influence the return of oestrus and ovulation: the importance of this effect is larger in freely nursing cows than in cows suckled twice a day.

The veal sucking stimulates the production of oxytocin by the cow, which is responsible for an increase in milk production and, as already mentioned for calves, has an "anti-stress" effect for the animal (Lupoli et al., 2001).

1.3 Stress at weaning

Under natural conditions, the separation between cow and calf occurs gradually towards 8-10 months of age (Johnsen, Ellingsen, et al., 2015). It seems that natural rearing conditions, such as free ranging and feral herds of cattle, give both cow and calf a high degree of welfare (Krohn, 2001). On the contrary it is well documented that weaning in dairy rearing systems causes a lot of stress (Flower & Weary, 2001; Hudson & Mullord, 1977; Lidfors, 1996; Weary & Chua, 2000). After the mother-young bond has been established, both cows and calves show increased vocalisation and

activity levels after separation and a period of weight loss is also often observed, accompanied by slower intakes of food and water (Flower & Weary, 2001; Weary & Chua, 2000). This pronounced behavioural and physiological response is a evident welfare problem, and can be considered an important production challenge for the livestock industry (Weary, Jasper, & Hötzel, 2008). This period of stress, however, does not last more than 3-4 days (S. Fröberg et al., 2011).

1.3.1 Effect of weaning on cow and calf behaviour: influence of cow-calf bond at the time of separation

To analyse how much the time of contact between cow and calf affects the stress at separation, several studies have been carried out in which different ways of separation, according to the age of the calves, have been compared.

Weary & Chua (2000) studied the effect of separation at 6h, 1 day and 4 days after birth. They found that cows and calves showed vocal and other behavioural responses to all types of separation, but they also showed that cows separated from their calves later after birth call with a higher fundamental frequency. This provides the evidence of a stronger behavioural response to separation at older ages than when separation occurs after birth.

Flower & Weary (2001) analysed the effect of separation at 1 day and 2 weeks of age. They observed an increased response as vocalization and movements to separation by both cows and calves when the calves were separated from their mother at 2 weeks rather than at one day of age.

Hudson & Mullord (1977) showed that separation is distressful for cows even if they have been allowed only a very short period (at least 5 minutes) of contact with the calf.

Lidfors et al. (1996) investigated the effect of separation immediately and 4 days after birth. They observed that cow and calf showed a higher activity immediately post-partum if kept together, and the separation after 4 days influences both activity, vocalisation and rumination of the cows and activity and oral behaviour of the calves. They reported that cows separated after 4 days ruminated less often then cows separated immediately after calving.

So weaning, even after a minimum contact, induces an increase of vocalization and agitations in the cows and increases blood cortisol levels in the calves. On the other hand, calves suckling their mother tend to spend less time on non-nutritive oral activities after weaning, such as tongue-rolling, licking or suckling other calves or objects, especially if they had suckled their dam during the first

two days of life (Veissier et al., 2013). Fröberg et al. (2011) found that the weaning procedure was more stressful for calves suckling than calves artificially reared, as shown by less ruminating and more vocalization and movements, but anyway they displayed less stereotypies. In general it has been seen that the incidence of cross-suckling decreases with increasing hay and concentrate consumption, solid food intake has been suggested to provide a replacement stimulus to suckling (Fröberg et al., 2011).

Delayed separation provides advantages also in terms of the development of social behaviour and this can compensate the increase behavioural response to separation (Flower & Weary, 2001).

1.3.2 Ways of reducing stress at separation

The moment of weaning in commercial systems imposes many stressors simultaneously, such as early separation from the dam, the change of diet from milk to solid, the introduction to new social partners and to a new environment. All these factors can negatively affect the animals and their combination accentuates the distress response. Therefore, modifying or separating these factors could decrease the weaning response. Weary et al. (2008) suggested reducing weaning response by preventing calves from nursing before weaning and then separating them from mothers a few days later. The aim of this practice is to allow the young animal to achieve nutritional independence before separating from the dam. They also propose to increase the intake of solid feed before weaning, increase the weaning age, increase the time of separation from the mother during the day and make the solid diet more attractive.

The first issue with cow-calf systems is the loss of weight gain of calves at weaning as a consequence of the premature separation compared to the natural one, which may cause more stress in free contact systems. Johnsen et al. (2016) suggested that half-day contact could reduce this factor because animals get used to being separated. Loberg et al. (2008) proposed a two-step weaning: 2 weeks before weaning calves were fitted with a nose-flap which prevented them from suckling while they were kept together with the cow. These calves shown less stress at weaning than calves separated simultaneously, in accordance with the previous study of Haley et al. (2005). In term of weight gain, Loberg et al. (2008) found no differences between the experimental groups so they concluded that the presence of the cow could only influence the weight gain of calves during the period of drinking milk and not afterwards.

Another gradual method of weaning is the “fence-line contact”, that is the separation of calves from their mother which allows the visual and auditory contact but not the physical contact. Price et al. (2014) shown that postweaning cumulative body weight gain was greater for beef calves separated with fence-line from mother on pasture than calves totally separated: in the first 2 weeks they gained 95% more weight than other groups of calves and they were still heavier at 10 weeks. They concluded that fence-line contact for 7 days after weaning reduce behavioural signs of distress and minimizes weight losses.

These results indicate that both cow and calf experience greater distress with later separation. However, later separation confers health benefits to both the calf and cow. Judging the best date for the animal welfare will require a balancing of these factors (Weary et al., 2008).

1.4 Impact of natural suckling on milk production

The effects of natural suckling on milk production have not yet been studied deeply. Different suckling methods were analysed and it was found that suckling *ad libitum* causes a clear loss of milk as cows strongly reduce the release of oxytocin and prolactin at milking (de Passillé et al., 2008). In fact generally the response of oxytocin to nursing is greater than to milking, so reducing the frequency of nursing could limit these suppressive effects on milk ejection during machine milking (Lupoli et al., 2001). De Passillé et al. (2008) allowed nursing to occur after milking rather than before milking to examine if it would prevent the reduction in oxytocin secretion during the milking, but they found that suckling also inhibits its release and consequently reduces milk ejection. On the contrary, Lupoli et al. (2001) have reported that a lower frequency of nursing reduces the suppression of oxytocin release during machine milking.

Some authors investigated the influence of the natural suckling on the total milk production corresponding to the milk yield at milking + calves milk intake. Fröberg et al. (2007) found that milking once daily and suckling twice-daily increased saleable milk output and they suggested that the increased milk production of cows nursing a calf could results from hormonal changes. The higher milk production is due to the calf teat stimulation and the better udder emptying if calves suckle the residual milk after milking (Sandoval-Castro, Anderson, & Leaver, 2000). More frequent udder emptying in early lactation stimulates the development of the milk secreting cells (Hale, Capuco, & Erdman, 2003). Mendoza et al. (2010) found no substantial differences in terms of total milk production between cows suckled twice daily and cows non-suckled when taking into

consideration that the difference in machine-milked milk yield between the treatment groups is similar to the amount of milk consumed by the calves.

So, taking into consideration calf's milk ingestion, it can be affirmed that suckling system can lead to a similar or greater milk production than non-suckling system, but the limit is that cows retain most of the milk during the milking in order to reserve it for the calf (Efsa et al., 2009). Fröberg et al. (2007)'s opinion is that cows retain milk at milking-machine because calves suckle immediately after. Suckling system would not only influence the amount of milk produced, but also its chemical composition, since the calf only takes a portion of the milk produced. Johnsen et al. (2016) reported that the milk fat content, and more particularly the polyunsaturated fatty acid content, of milk during the lactation period is lower than that of a cow not suckled. It can then be assumed that the milk fat content of the milk drunk by the calf is higher. Furthermore, if calves suckle the cow after milking they consume the residual milk, which has a higher fat content than the machine-milked milk and consequently the fat content at next milking is reduced as a carry-over effect. Tesorero et al. (2001) have shown that in a restricted suckling system where calves suckle before milking, the saleable milk yield and fat content increased, compared to non-suckling before milking. In that situation, calves suckle the first milk portion that has the lowest fat content as milk fat content increases during milking. The protein content does not seem to be impacted by the practice.

In conclusion, it has been suggested that a combination of milking and suckling can increase both saleable and total milk yield in low-producing dairy cows if the activation of milk ejection may be difficult without calf stimulation (Fröberg et al., 2007). Contrarily in dairy cows selected for milk production, machine-milked milk yield can be reduced by suckling but it could be a viable alternative for raising calves because they can achieve a higher milk intake than is normally recommended in systems with artificial rearing (Mendoza et al., 2010).

1.5 Technical and economic implications and work organization for the farmer

The choice to let the calf suckles its mother has significant impacts on the farmer workload and the organisation of the farming system. From an economical point of view, natural suckling can lead to milk production decreases in the milking parlor, although the choice of an adequate system, often corresponding to controlled suckling, limits and even prevents milk losses. On the other hand, suckling system can contribute to a better growth and development of replacement heifers and animal sold for fattening (Efsa et al., 2009).

Apart from giving the animals increased opportunity to express their natural behaviour, compared to artificial rearing system, natural suckling system may save labour by allowing the cows to take care of their offspring (Fröberg et al., 2011). It is however possible that, for some farmers, the choice of this practice requires a spatial reorganisation of livestock buildings in order to bring the calves closer to mothers and to avoid health risks linked to the vulnerable immune system of young calves (Johnsen et al., 2016). In the study by Wagenaar & Langhout (2007), the farmers of the 4 organic farms have adapted well to the rearing system of the heifers, despite some reluctance due to the loss of marketable milk. According to Grøndahl et al. (2007), the practice is appreciated by the farmer, easy to manage and does not require additional work. They also consider that natural suckling should be possible also for larger herds as the greatest benefit is the easy calf feeding system that can guarantees good calf health.

In the contest of one daily milking, the work required is lower while maintaining an acceptable production thanks to the separation between the calf and its mother at night (Pomiès et al., 2010). Although milk consumption by the calf is often considered a negative aspect, it can also be regarded as an investment in growth and development of a future dairy cow (Wagenaar, 2009).

Apart from the effect on the calf, if a restricted suckling system with once-daily milking is to be economically feasible, the amount of saleable milk ought to be comparable with the amount of milk obtained in artificial rearing systems with once-daily milking less the cost of milk or milk substitute for the calf (Fröberg et al., 2007).

1.6 Suckling systems for dairy calves within the EU context

Studies conducted on the subject reveal significant effects of this practice on many factors that can affect the production system as a whole. The quality and quantity of the characteristic of milk and ADG of calves can be significantly modified, the health of the cow's udders and possibly also the long-term calf's health. Nutrition and mother-child interactions contribute to establishing a strong bond, beneficial for the development of the animal, but which accentuates the number of vocalizations at weaning and therefore its difficulty. The choice of practice also saves time and reduces the workload.

Despite this, the suckling system is a new subject of research, it still requires many studies and especially in Europe will be implemented several research projects in order to analyse all aspects of this practice. In fact, the development of dam’s milk immunoglobulins in relation to calves’ health

state has not been investigated, yet. Furthermore, economic consequences of different calf rearing systems including differences in the amount of milk sold have not yet been evaluated.

The majority of consumers express opposition to the practice of early separation of dairy cows and calves after birth in Germany and the United States (Busch et al., 2017). The number of organic farms practising dam rearing is unknown in most European countries (Kälber and Barth, 2014). In Norway and Sweden 18% and 22% of the organic dairy farmers let the calves suckle for 1 to 13 weeks thus exceeding the mandatory period which is 3 days in Norway and 1 day in Sweden (Johnsen et al., 2016). In the Netherlands, 30% of the bio-dynamic dairy farmers let cow and calf together for 2 to 4 months. Although farms that have already implemented dam rearing systems are mainly organic farms, the majority of dairy calves on organic farms is reared with restricted milk amounts like 6-7 l/d over 3 months. This may lead to impaired growth and to welfare problems, e.g. increased mortality, intersuckling or hunger (Vasseur et al., 2010). Long-term benefits of increased milk feeding like a higher milk production in the first lactation have been reported (Shamay et al., 2005).

The project "ProYoungStock - Promoting young stock and cow health and welfare by natural feeding systems " is one of the projects initiated in the framework of the ERA-Net project CORE Organic Cofund.

The ProYoungStock project aims to investigate the (long-term) effects of natural rearing and feeding systems on health, animal welfare and profitability in order to solve current problems and find examples of good professional practice.

The objective of ProYoungStock is to collect, develop and assess natural feeding strategies to increase dairy livestock welfare, including health, in different agro-ecological and regulatory European contexts. This will be achieved by improving the rearing of calves pre-weaning and designing forage-based feeding strategies for heifers and adult cows, both fostering the animal's immune status and reducing the use of antibiotics and anthelmintics.

Another project of CORE Organic Cofund is "GrazyDaisy" which investigates how to manage mixed age groups of cows, including rearing calves with their dams, whilst maintaining a high level of health and a constant effort to minimize medication. It focuses on implementing resilient grazing strategies to improve animal performance and decrease impacts on the ecosystem on and around the farm. In particular it addresses the special challenges of keeping older and younger animals together on

grass. The project aims to encourage grazing and improve grazing management, rearing cows and calves together, and minimizing medicine use.

2. OBJECTIVES

The project involves the French National Institute for Agricultural Research (INRA), UMR Herbivores and the experiment took place in UE Herbipole (farm of Marcenat, France), a platform dedicated to herbivores reared in mountain areas. Since 2017, INRA is trying to develop farming systems that allow maintaining mother-calf contact until weaning, without affecting cow performances, milk quality and the growth of future heifers. The work started in 2017 with an experiment where a classic rearing system (Group 'Control') was compared to two different suckling experimental methods (Groups 'Before Milking' and 'After Milking') allowing a brief dairy contact between calves and mothers. The weak growth of calves 'After' (375 g/d) that consumed half the milk of calves in the other two treatments led to a premature end of this experimental treatment after eight weeks. Conversely, the growth of calves 'Before' was faster than that of controls (832 vs. 644 g/d until Week 8), in line with their higher milk consumption (7.6 kg/d) (Pomiès et al., 2018). The results obtained were not those expected: suckling before milking resulted in a high loss of milk collected at milking and the reunion of calves with mothers for 20 minutes before milking caused an increase in labor for farmers.

Starting from these results, in 2018 a new study has been made. The aim was to reduce the labour and milk losses: a classic rearing system was compared to a natural nursing rearing system (where cows and calves were separated only after the evening milking until the morning milking in order to allow calves to suckle all-day long). At the morning milking a higher production of milk was expected, as the time of separation of the cows from the calves was about 15 hours.

The aim was to study the impact of this practice on milk yield, milk composition, growth of calves and the animal behaviour and welfare.

3. MATERIALS AND METHODS

3.1 Experimental Research Centre

French National Institute for Agricultural Research (INRA) is Europe's top agricultural research institute and the world's number two center for the agricultural sciences. INRA is a national public scientific research institution under the dual aegis of the Ministry of Research and the Ministry of Agriculture.

The experiment took place at INRA Herbipole. Herbipole is an experimental unit dedicated to the rearing of herbivores in mountain areas, with a set of infrastructures adapted to new research questions, especially the sustainability of livestock systems. This platform is installed on three sites of the Auvergne-Rhône-Alpes Inter Center (Theix, Laqueuille and Marcenat), and offers an opening of the infrastructures to the research partners (national and international) and to the professionals of the breeding. The site of Marcenat is dedicated to researches on dairy cows.

The site of Theix, UMR Herbivore, is a joint research unit between INRA and VetAgro Sup (Institute of Higher Education and Research in Food, Animal Health, Agroscience and the Environment) and it conduct research on cattle and sheep and their production system. In particular it is focus on CH_4 emissions, animal welfare, feeds value, and product qualities.

3.2 Animals, Housing and Feeding Plan

The project was carried out between February and July 2018 at INRA, UE Herbipole, in the site of Marcenat, France. The experiment involved 28 cows with their calves that were monitored for 13 weeks after calving. Parturitions took place between the end of February and the end of April. The 14 Montbéliarde (Mo) and 14 Holstein (Ho) cows involved in this experiment were selected on the basis of their lactation rank, date of calving, milk index and sex ratio of calves. Two experimental groups (group 'Control' and group 'Mother'), composed of 7 Mo and 7 Ho cows each were made so that within each breed, the lactation rang, the date of calving and the milk yield (index for primiparous cows and total milk yield during the previous lactation for multiparous cows) were equivalent. At calving the animals were paired in the two groups. The 2 groups are conducted according to a "classic" farming protocol, in 2 parks of 14 places each. They did not change pen from before to after weaning.

In the *'Control'* group, the calves were separated from their mothers within the few hours after birth and were housed in individual pens, fed twice a day with milk from the tank for 7 days. Successively

they were placed in a collective park for 9 weeks and fed with bulk milk (up to 10 kg/day) and concentrate (up to 2 kg/day) by an automatic feeder (*Fig. 1*). The detailed calves' feeding plan is described in *Table 1*. Good quality hay was available *at libitum*. Weaning was made after 10 weeks when calves' live weight was about 100 kg. Then, calves were fed with concentrate (up to 4 kg/day) and good quality hay fed *ad libitum*.

(Fig. 1) Calves 'Control' collective park

In the *'Mother'* group, the calves spent 5 days after parturition in an individual calving pen, bedded with straw, with their mother in order to allow mother-calf attachment (*Fig. 2*). Cows were taken to the parlour to be milked twice daily at approximately 07:30 and 16:30. After this period, both cow and calf were moved to the collective park for 9 weeks. Cow-calf contact was allowed during the day: calves had free access to the stabling of mothers for suckling between the return of the morning milking and the departure for the evening milking. During the night they were separated from the mothers and housed in the calves' collective park, next to the stabling of the mothers. There, calves were isolated in their park with mulched soil and covered shelter (*Fig. 3*). They could see their mother, at least at the level of the separation of the 2 parks (*Fig. 4*). Calves suckled the cows freely during the day and had access to concentrates and good quality hay *at libitum* 24h a day. Weaning was made after 10 weeks when the calves' live weight was about 100 kg. Then the calves were fed with concentrate (up to 4 kg/day) and good quality hay *ad libitum*.

(Fig. 2) Individual calving pen for the 5 first days after parturition

(Fig. 3) Calves 'Mother' collective park

(Fig. 4) Calves' collective park next to the stabling of the mothers

Table 1 *Calves feeding plan*

Group	Weeks	1	2	3	4	5	6	7	8	9	10	13	14	15
Control	Milk (kg/d)	6	7	9	10	10	10	9	7	5	3	**→weaning**		
	Concentrates (kg/d)	0	0	0.2	0.4	0.6	0.9	1.2	1.5	1.8	2	~2.00	~2.00	~2.00
	Hay	0	*Ad libitum*									*Ad libitum*		
Mother	Suckling period	24/24h	From morning milking to evening milking									**→weaning**		
	Concentrates (kg/d)	0	*Ad libitum*									~2.00	~2.00	~2.00
	Hay	0	*Ad libitum*									*Ad libitum*		

All cows received a complete mixed ration of fodder, concentrate and minerals until the beginning of the grazing period in early May. During the grazing period, the 2 groups were separated in 2 adjacent plots, with inversion every morning. During the day, the calves 'Mother' were allowed to graze with their mothers while the calves 'Control' stayed inside. (Before setting on the grass, a diurnal release of calves and mothers for habituation on a fenced area common has been done for a few days).

3.3 Measurements and data collection

During the experiment, measurements and analyses concern milk production and composition, cow's body weight (BW) and Body Condition Score (BCS), calves' growth, individual quantity of milk and concentrates ingested by calves in the group 'Control', quantity of concentrate ingested by calves group 'Mother', health events of cows and calves and different types of behavioral observations on all the animals involved in the study.

3.3.1 Cows performances:

Milk yield of each cow was recorded at the milking parlour twice a day. Milk fat and protein content were measured on individual milk samples from 4 consecutive milkings per week and somatic cell count (CCS) from 2 consecutive milkings per week. These measurements allowed to calculate the individual milk production daily, weekly and at the peak of lactation, as well as the individual weekly milk composition.

Body weight (BW) and body condition score (BCS; 0-5 scale) of each cow was measured at calving, once a month and at weaning by two specialized technicians following the body condition score method (BCS). The body condition score is a visual and tactile method that gives the possibility to evaluate the body fat reserves of an animal, based on a numerical score scale which varies according to the type of animal. For dairy cattle a 5-point scale is used with 0.25-point increments. The evolution of the BCS between two dates gives an indirect estimation of body energy balance. These measurements allowed to draw individual BW and BCS curves for each lactation. All the health events were recorded and ranked into two classes:

- *Type 1* for reproductive disorders (metritis, retention of placental membrane, ovarian cysts and vaginitis);
- *Type 2* for all other types of disorders (mastitis, milk fever, lameness, conjunctivitis, keratitis etc.).

3.3.2 Calves performances:

Calves were weighted at birth and once a week until the end of experiment in order to control the weight gain and to keep the health and the good growth of the animals under control. All the health events (diarrhoea, respiratory problems, etc.) were recorded and ranked into two classes:

- Respiratory diseases (runny nose, coughing etc.);
- Other types of disorders (umbilical infection).

Calves 'Control': a daily registration of individual milk and concentrate intake was made by the automatic milk and feed dispenser, respectively.

Calves 'Mother': starting from the 5th week of the experiment and for a period of 6 weeks, milk intake was estimated the days of weighing. All calves were weighted once before the reunion with the mothers and once after 1 hour, to allow them to suckle the necessary amount. Starting from the 4th week, one day per week, a fixed amount of concentrates was given to calves and the following day this amount was weighted in order to calculate the quantity consumed by all calves during the day.

3.3.3 Behavioral observations:

I. MOTHER-YOUNG BOND

- "*<u>After Calving</u>*": *Monitoring the formation of mother-young bond*

The first 5 days after each calving, mother-young bond formation was monitored. During the day, in the period after the morning milking and before the evening milking, an observer verified that the calf had well learned to suck and if the mother had a maternal behavior towards him (licking, sniffing etc.). In particular, during the first 2 days after birth, the observed verified that the calf started to suckle to be sure that it had taken the colostrum promptly. Otherwise the observer had to stimulate the calf to start suckling.

- *"<u>Morning Reunion</u>"*: *Monitoring the maintenance of mother-young bond*

From the beginning of March to the start of the grazing period (beginning of May) every Wednesday of each week, a behavioral observation was carried out during the first hour after the morning milking. The aim was to monitor the behavior of calves and cows during the first reunion of the day. To realize the observation, every Tuesday the animals (both calves and cows) were painted with an identification number. The choice of the number was based on the calving order: each cow-calf couple has been assigned a number (from 1 to 14) to allow a rapid identification of the couple. The observations were made by 2 expert observers and using 2 cameras placed on the collective park. The animals were painted on their flanks and back with large numbers to allow observers and cameras to recognize them easily (*Fig. 5*).

(Fig. 5) Observer monitoring the 'Morning reunion'

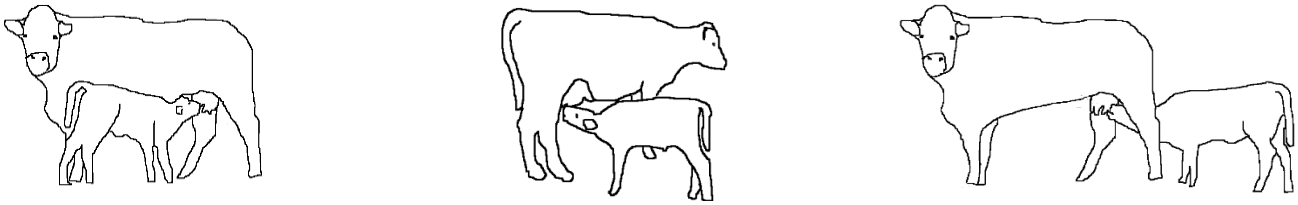
The aim of "Morning Reunion" observation was to report all the suckling behaviors of the calves, for a period of 1h. the following behaviors were registered:

-*Reunion latency:* time (sec) passed since each calf entered in the cows' park until it reached its mother;

- *Successful suck attempts from Mother and from Other cows:* number of time and duration (sec) of every successful suck attempt performed by each calf to its mother or another cow.

- *Refused suck attempts from Mother and from Other cows:* number of time and duration (sec) of every refused suck attempt performed by each cow to its calf or another calf.

- *Type of suckling position:* revers parallel position, anti-parallel position (45°), behind position. (*Fig. 6*).



(Fig. 6) a) Reverse parallel suckling position; b) Anti-parallel suckling position; c) Behind suckling position

II. BEHAVIOUR OF CALVES DURING THE DAY

- "<u>*During the day*</u>": *Behavior of calves 'Control' and couple mother-calves 'Mother' during the day*

Starting from 4th of April, in addition to the Wednesday morning observation ("*Morning Reunion*", 1h after the morning milking), supplementary observations were made during the day. The observations concerned both the Mother-calf couples and all the calves Control.

The aim was to study if the behavior of calves with mothers was similar or different from the behavior of calves without a mother. To do this, each calf of the group 'Control' has been paired with a calf of the group 'Mother' with the corresponding identification number.

In order to observe the calves 'Control'-calves 'Mother' couples at the same time, 2 expert observers were employed (one in charge of observing calves 'Control' and one in charge of observing calves 'Mother') and 3 cameras positioned respectively on the collective park of calves Control, collective park of cows 'Mother' and collective park of calves 'Mother'.

The type of observation realized is called "Focus Observation": each individual is observed for a continuous period of time (10 minutes) during which all the activities performed and the duration (sec) of each one are recorded. The activities recorded are the following:

Classes of behaviours observed	**Types of activities**
Individual activities:	- *Feeding* (eat solid feed and drink water) - *Drinking milk* (automatic milk dispenser, for calves Control*)* - *Ruminate* - *Exploration* - *Self-grooming* - *Walking* - *Observing* - *Locomotor play* (running, jumping and kicking in the air) - *Vocalization*
Positive social interactions with other calves:	- *Approach to a calf* - *Get away from a calf* - *Sniffing* - *Licking* - *Social game*
Negative social interactions with other calves:	- *Pushing* - *Kicking* - *Head-butt*
Social interactions with Mother and Other cows (only for calves Mother):	- *Approach to mother or another cow* - *Get away from mother or another cow* - *Successful suck attempts* (position, duration and number of attempts) - *Refused suck attempts* - *Sniffing* - *Licking* - *Social game*

The notation consisted in observing at the same time each pair of calves, following a pre-established weekly order. Each pair was observed for 10 continuous minutes and during this period all the activities done by the observed animal were reported. In addition, a scan was performed every 30 seconds, within these 10 minutes, in order to indicate the closest companion (calf or cow) to the

subject taken into consideration: the aim was to evaluate the social relationships that have been created between the animals of the same park.

With the aim of analyzing how the presence of cows affects the behavior of calves, we registered the *positive* and the *negative social interactions* between animals. With positive social interaction, we meant all the behaviors concerning the contact with another similar, such as *licking, sniffing, social game* etc. All behaviors which provide dominance or competition in moments related to time of eating or choosing a place to lay are considered negative social interactions (*pushing, kicking and head-butt*).

The program of observations was divided into two periods: the first period started at 9:00 am and the second period started at 13:00 pm. The duration of the observations varied from week to week depending on the number of animals present in the parks (10 minutes for each calf present), generally had a duration of 1:30 and 2:30 hours. Overall, each individual was observed for a period of 20 min/d (10 min in the morning + 10 min in the afternoon).
To reduce the 'observer effect', the one who had observed the calves 'Control' in the morning observed the calves 'Mother' in the afternoon and this order was inverted every week.

III. WEANING DISTRESS

The weaning was done when the calves reached a BW of about 100 kg. The program was designed in such a way as to be able to wean at the same time a similar number of calves belonging to both groups. Overall, 3 weaning periods were realized: the first group took place on May 15th (6 calves 'Mother', 5 calves 'Control'), the second on May 29th (5 calves 'Mother', 6 calves 'Control') and the last group was weaned on June 26th (3 calves 'Mother', 3 calves 'Control').
The day of weaning, the calves of each group were removed from their park and moved to a new park. For the weaning week, the two groups were kept separate: the group of calves 'Mother' have been housed in a separate park from calves of group calves 'Control'. This was done with the aim of not adding other stress factors in addition to the separation from mothers, the elimination of milk from the diet and the change of environment which can subsequently influence the change of behavior. Starting from the second week after weaning, the two groups were reunited until the end of the study. During the week in which the weaning was done, two types of behavioral observations were done. They concern the percentage of animals mooing and the number of vocalizations.

- *"Percentage of animals mooing during the week after weaning"*

The stress level of cows and calves was evaluated by monitoring vocalizations and behavior during the day. The days of observations were Monday (day before weaning, D0), Tuesday (weaning, D1), Wednesday (D2), Friday (D4) and the following Monday (D7). Observations was focused on cows and calves weaned (group 'Mother' and group 'Control').

Observations of cows:

- *During the day* (period between the two-daily milking): registration of the animals who vocalize (0 nothing, + time to time, ++ frequently)
- *On the way to the milking parlour*: registration of the agitation of the cows (0 nothing, + vocalize, ++ cow stops, turns around)
- *During the milking*: registration of the agitation of the cows (0 nothing, + vocalize, ++ kicking)

Observations of calves:

- *During the day* (period between the two-daily milking): registration of the animals who vocalize (0 nothing, + time to time, ++ frequently)

- *"Number of animals' vocalisations during the week after weaning"*

The number of vocalizations during the 30 minutes following the morning milking was recorded. The days of observations were Tuesday (weaning, D1), Wednesday (D2) and Friday (D4).

The aim of this type of observation was to observe at the same time the behavior of mothers and calves. To do this, at the end of the morning milking, two observers were instructed to report at the same time the number of vocalizations made by the mothers and by the calves. The recordings were done for both groups 'Mother' and 'Control': the cows and respective calves were observed for 30 minutes in the group 'Mother' then in the group 'Control'.

3.4 Statistical analyses and calculations

The milk production and composition of cows used in statistical analyses correspond to the average weekly data of each individual cows during the first 8 weeks of lactation. For calves' performances, we calculated the ADG at 4 weeks, at weaning, 3 weeks before and after weaning as follow:

$$ADG\ 4\ weeks\ (g/d) = \frac{BW\ at\ 4\ week\ of\ age\ (kg) - BW\ at\ bith\ (kg)}{28\ (d)} * 1000$$

$$ADG\ at\ weaning\ (g/d) = \frac{BW\ at\ weaning\ (kg) - BW\ at\ bith\ (kg)}{age\ at\ weaning\ (d)} * 1000$$

$$ADG\ 3\ weeks\ before\ weaning\ (g/d) = \frac{BW\ at\ weaning\ (kg) - BW\ at\ 3\ weeks\ before\ weaning\ (kg)}{21\ (d)} * 1000$$

$$ADG\ 3\ weeks\ after\ weaning\ (g/d) = \frac{BW\ at\ weaning\ (kg) - BW\ at\ 3\ weeks\ after\ weaning\ (kg)}{21\ (d)} * 1000$$

The animal performance data (cows and calves) were analysed with a Mixed model (PROC MIXED, SAS 9.4 version, 2004, SAS Institute Inc., Cary, NC) that included the experimental group (Mother or Control), the breed (Holstein or Montbéliarde) and the interaction (group * breed) as fixed effect. The random effect was the couple cow-calve. For cows, the statistical model also included covariates: the calving date and the parity (primiparous or multiparous) for all variables and the dairy index for milk yield, the calving weight for body weight and the BCS at calving for BCS. For calves, the model included the sex (male or female), the age at weaning and the birth weight as covariates for all variables except ADG at 4 weeks. SCC data were converted into decimal logarithm for the statistical analysis but results are presented in the tables as count means (x10^3 cells/mL) in the text, tables and figures. Means reported in the tables are the adjusted by the mixed model. The health events (counting data for cows and calves) were analysed with a khi2 test.

All the data related to behaviours recorded once a week for each individual calf were averaged during the entire observation period. We also calculated a selectivity index as follows:

$$Selectivity\ (S) = \frac{V}{D}$$

where:

$$V = \frac{n°of\ times\ cow\ accepts\ own\ calf}{n°\ times\ accepts\ own\ calf + n°\ times\ refuses\ own\ calf}$$

$$D = \frac{n°of\ times\ cow\ accepts\ other\ calves}{n°\ times\ accepts\ other\ calves + n°\ times\ refuses\ other\ calves}$$

Data related to behavioural were analysed with the MIXED procedure of the SAS that included the experimental group (Mother or Control), the breeds (Holstein or Montbéliarde) and the interaction (group * breed) as fixed effects. The date as the repeated effect.

For all analyses, the differences were considered significant below the threshold P value of 0.05 and a trend was considered at $0.05 < P < 0.10$.

4. RESULTS

4.1 Animals performances results

4.1.1 Cows' performances:

The average cow's performances from week 1 to week 8 after calving are presented in *Table 2*.

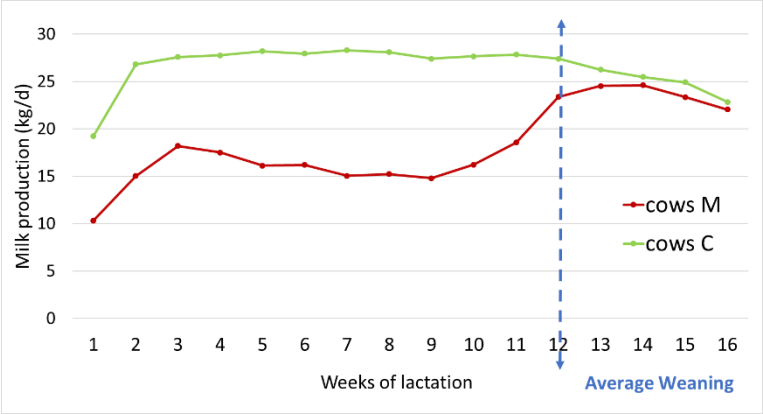
Table 2 *Milk production and composition of cows and body weight and BCS of cows according to the group and breeds (Week 1 to 8 after calving)*

	Cows Mother		Cows Control		*P*			
	Ho (n=7)	Mo (n=7)	Ho (n=7)	Mo (n=7)	Group	Breed	Group*Breed	Covariates[1]
Milk production (kg/d)	14.6	16.6	29.0	24.8	***	ns	+	ns
Milk fat content (g/d)	30.1	30.2	40.9	36.8	***	ns	ns	-
Milk protein content (g/d)	31.2	30.9	29.0	30.3	*	ns	ns	-
Milk SCC (x10³/mL)	5.06	4.73	4.76	4.55	ns	ns	ns	-
Weight at weaning	645	630	628	634	ns	ns	ns	***
BCS at weaning	1.45	1.43	1.72	1.56	ns	+	ns	**

***P<0.001; ** P<0.01; * P<0.05; + P<0.10; ns P≥0.10

[1]: dairy index for milk production; BW at calving for BW; BCS at calving for BCS

I. Milk production:



(Fig. 7) Average daily milk production (kg/d/cow) during the 16 first weeks of lactation

Starting from the first week of lactation, the milk yield is lower in the group 'Mother' than in the group 'Control' (-8.9 kg/d; raw data; *Fig.7*). This difference increases from week 1 to 8 until reaching a maximum at the 8th week of lactation (-12.9 kg/d; raw data; *Fig. 7*). During the first 8 weeks, the difference in milk production between the 2 groups was significantly different (-11.3 kg/d; P<0.001; *Table 2*). Even if the milk yield of the two breeds was similar, the difference between 'Mother' and 'Control' groups tended to be higher in Holstein than in Montbéliarde breed (-14.6 kg/d *vs.* -8.3 p<0.01).

Starting from the 9th week, when the first weaning took place, the difference decreases until the 13th week of lactation. From the 14th week, when all the calves have been weaned, the loss of production stabilizes and becomes minimal between the two groups (-1.1 kg/d; raw data; *Fig.7)*.

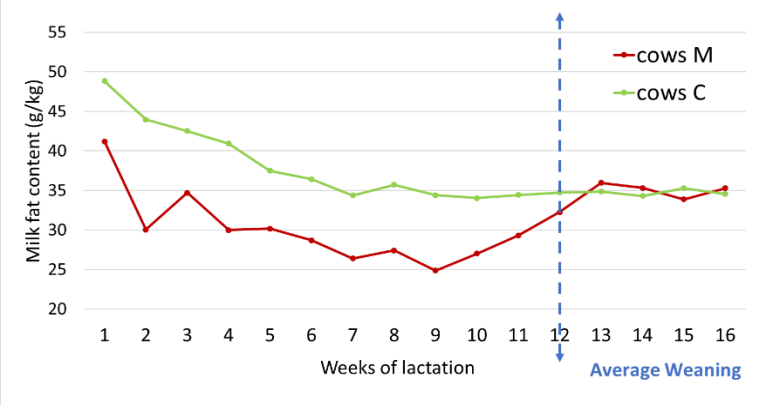
For milk production, the covariables used were not significant.

II. Milk composition:

Milk fat content: During the first 8 weeks of lactation, milk fat content was significant lower in the group 'Mother' than in the group 'Control' (P<0.001; *Table 2*). On average, the difference between the two groups was of 9.3 g/kg (raw data; *Fig. 8*). The milk fat content was similar in Holstein and Montbéliarde breeds (P>0.01) and the effect of the treatment was the same in the two breeds (interaction breed*group ns, *Table 2*).

When all the calves have been weaned (starting from the 12th weeks of lactation) until the end of the study, cows of group 'Mother' have increased their average milk fat content up to that of the cows 'Control' (-0.35 g/kg; raw data; *Fig. 8*).

We found no significant difference in milk fat content linked to the parity and the calving date.



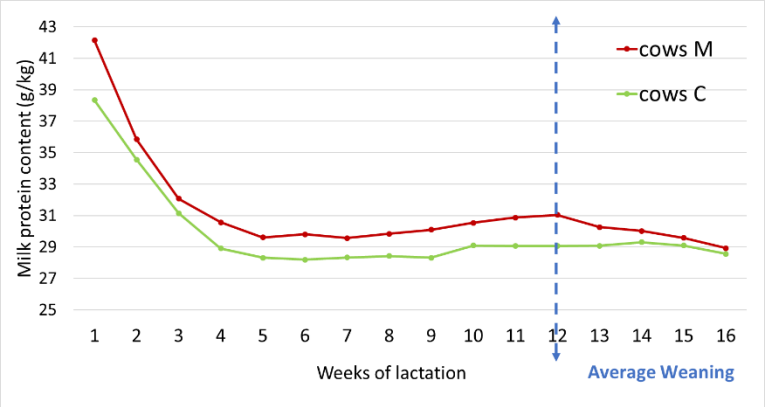
(Fig. 8) Average milk fat content (g/d/cows) during 16 weeks of lactation

Milk Protein Content: Milk protein content's from week 1 to week 8 was significantly higher in the group 'Mother' than in the group 'Control' (+0.9 g/kg, P<0.05; *Table 2*).

From the 13th weeks after the end of weaning, the difference between the two groups begins to decrease: the milk protein content of cows 'Mother' decreases to reach the milk protein content of the cows 'Control' (respectively, 28.9 g/kg *vs.* 28.5 g/kg at the 16th week; raw data; *Fig. 9*).

The milk protein content was similar in Holstein and Montbéliarde breeds (P>0.01) and the effect of the treatment was the same in the two breeds (interaction breeds*group ns, *Table 2*).

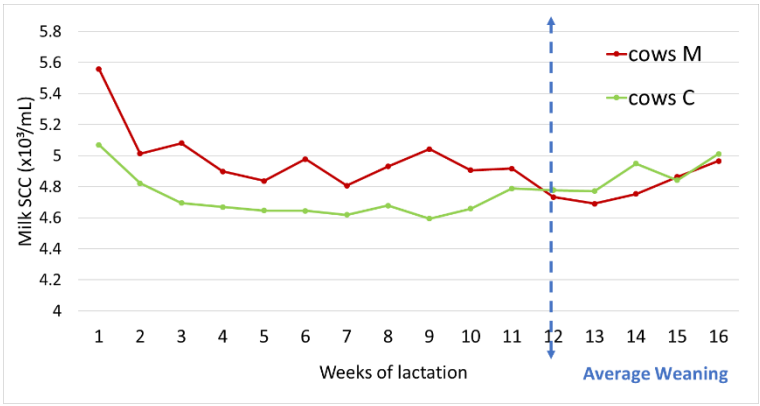
We found no significant difference in milk protein content linked to the parity and the calving date.



(Fig. 9) Average milk protein content (g/d/cows) during 16 weeks of lactation

Milk somatic cells count: The milk somatic cells count during the first 8 weeks of lactation was not significantly different between the 2 groups (P>0.10; *Table 2*). Even if in average the SCC was slightly higher in group 'Mother' than in group 'Control' from week 1 to week 10 (respectively 5.0 x10^3/mL vs 4.7 x10^3/mL; raw data; *Figure 10*). Starting from the 13th week, the average difference between the two groups tended to decrease.

We found no significant difference in milk somatic cells count linked to the breed, the parity, the calving date and the interaction group*breed was not significant.



(Fig. 10) Average milk somatic cells count (x 10^3/mL) during 16 weeks of lactation

III. Body weight and body condition score at weaning:

The BW and BCS of cows at weaning were not significantly different between the two groups (*Table 2*). For the BW at weaning we found no differences according to the breed, the parity and the group*breed interaction. The BW at weaning was significantly correlated with the BW at calving (P<0.001) and the BCS at weaning was significantly correlated with the BCS at calving (P<0.01).

IV. Health events:

The occurrence of *type 1* health events (metritis, retention of placental membrane, ovarian cysts and vaginitis) was not significantly different between the two groups (16% of cows 'Control' affected vs 36% of cows 'Mother' affected; P=0.19).

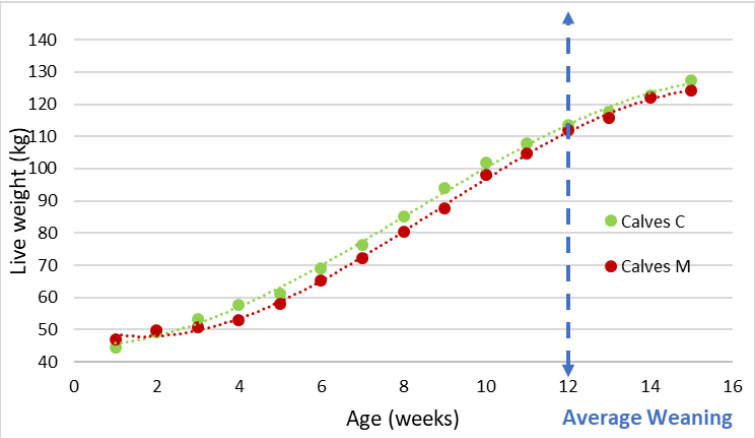
The occurrence of *type 2* health events (mastitis, milk fever, lameness, conjunctivitis, keratitis etc.) tended to be lower (P=0.09) for cows 'Mother' (14%) than for cows 'Control' (46%, mainly lameness and milk fever).

4.1.2 Calves' performances:

The study of the calves' performances was carried out with reference to the growth, mainly characterized by the average daily gain (ADG).

We considered the critical periods that can influence the growth trend of animals. The aim was to compare the growth performance of both groups (calves 'Mother' and calves 'Control').

I. *Calves growth:*



(Fig. 11) Growth curves of the two groups of calves

The *Figure 11* shows the average growth of the two groups of calves from birth until the 15th week of life. The growth of calves of the 2 groups followed the same trend, with an acceleration starting from week 5 and a slow-down after weaning.

Table 3 *Main characteristics of calves' growth according to the group and the breed.*

	Calves Mother		Calves Control		*P*			
	Ho (n=7)	Mo (n=7)	Ho (n=7)	Mo (n=7)	Group	Breed	Group*Breed	Covariates[1]
ADG 4 weeks after birth (g/d)	286	576	512	733	ns	+	ns	ns
ADG at weaning (g/d)	838	1037	869	1028	ns	**	ns	**
ADG 3 weeks before weaning (g/d)	1326	1490	1126	1245	**	ns	ns	ns
ADG 3 weeks after weaning (g/d)	402	410	567	375	ns	ns	ns	ns
Delta ADG (g/d)	-924	-1080	-558	-870	*	ns	ns	+
Weight at weaning (kg)	107	121	108	121	ns	**	+	*

***P<0.001; ** P<0.01; * P<0.05; + P<0.10; ns P≥0.10

[1]: sex, age at weaning and birth weight for all variables; sex and birth weight for ADG 4 weeks

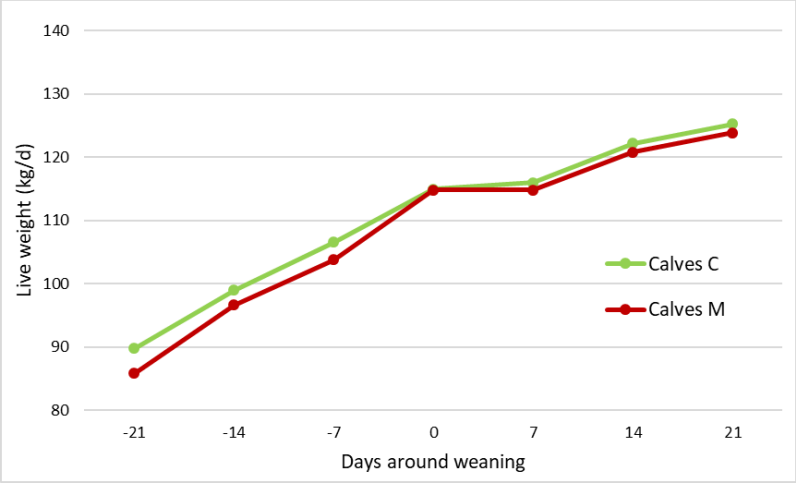
- *ADG at 4 weeks after birth and until weaning*:

We found no significant difference between the two groups. As show in *Figure 11,* the growth of the two groups follows the same trend. Montbéliarde (Mo) calves tend to grow faster than Holstein (Ho) calves at 4 weeks after birth (respectively 655 g/d *vs.* 399 g/d; adjusted data, P=0.07) and ADG until weaning of Mo calves was significantly higher than that of Ho (respectively 1033 g/d *vs.* 854 g/d; adjusted data, P<0.01).

We found no significant difference in ADG at 4 weeks after birth linked to the other parameters (sex, BW at birth and group*breed interaction), *(Table 3).* On the contrary, the ADG until weaning of male was significantly higher than ADG of female (P<0.05, 994 g/d *vs*. 890 g/d; adjusted data). BW at birth and age at weaning had a significant positive effect on ADG at weaning, P<0.01 (*Table 3*).

- *ADG around weaning:*

Figure 12 focused on the growth of calves around weaning. Calves 'Mother' at 21 days before weaning had a lower average BW than calves 'Control', but at weaning, they reached the same weight than calves 'Control'. After weaning the two curves follow a similar trend: a decrease during the first week after weaning and then a constant increase in the two following weeks.



(Fig. 12) Calves growth (kg/d) around weaning (3 weeks before and 3 weeks after)

During the 3 weeks before weaning, calves 'Control' had a significantly lower ADG than calves 'Mother' (P<0.05; -223 g/d; adjusted data). We found no significant effect of the other parameters (breed, sex, birth weight, age at weaning and group*breed interaction). *(Table 3)*

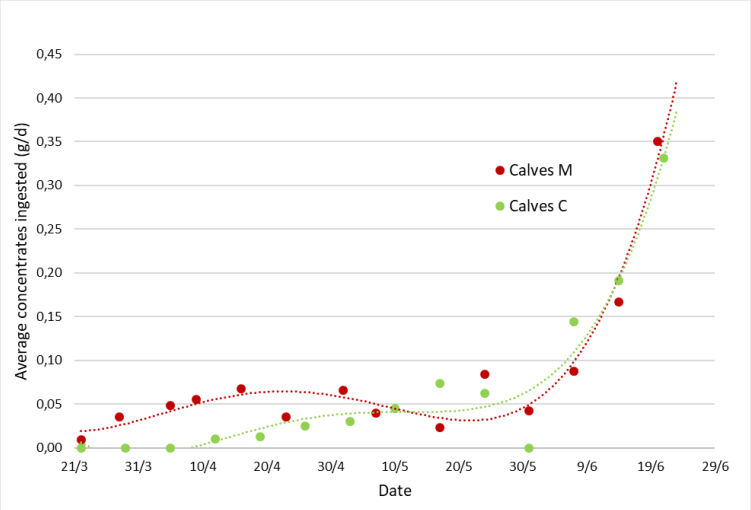
During the 3 weeks after weaning, the ADG slowed-down and no more difference between groups was evidenced (P>0.01). Nevertheless, the decrease in ADG after weaning (Delta ADG around weaning) was significantly higher for 'Mother' calves than for 'Control' calves (P<0.05). Three weeks after weaning, the effects of the breed, the sex, the birth weight, the age at weaning and group*breed interaction were not significant. The Delta ADG around weaning tended to be negatively affected by the age at weaning (P=0.07; *Table 3*).

As regard weight at weaning, we found a significant difference between the Ho calves and the Mo calves (respectively 107 kg *vs*. 121 kg, P<0.01; adjusted data). Also the age and the sex of calves affected the weight at weaning (P<0.05; *Table 3*).

II. *Control of calves' feed ingestion*

- Calves' concentrates feed ingestion:

Figure 13 shows the average daily concentrate feed ingestion of calves from both groups during the 10 weeks of measurements. Values reported correspond to the average quantity ingested considering all the calves present and not the proportion of calves ingesting concentrates (the number of calves present increased over time). The two curves have a similar and increasing trend (from 0.1 kg/d/calf to 0.35 kg/d/calf at 10th week; raw data). The average concentrates feed ingestion was similar in the two groups.

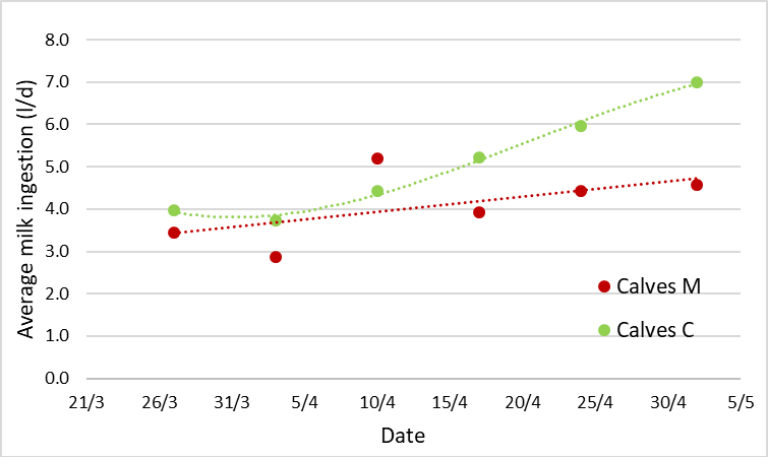


(Fig. 13) Average individual daily concentrates feed ingestion of total calves present (kg/d/calf)

- Calves' milk ingestion:

Figure 14 shows the average daily milk ingestion of calves from both groups during the 6 weeks of measurements. The values reported for calves 'Control' correspond to the total daily milk ingestion measured by the automatic milk feeder while the values reported for calves 'Mother' correspond to the estimation of the milk ingestion on the first suckling period in the morning. The milk ingested by the calves is significantly higher for the 'Control' calves than the 'Mother' calves but for the latter this quantity is largely underestimated.

The two curves have an increasing trend, in coherence with the increase of calves' age.



(Fig. 14) Average daily milk ingestion of calves during 6 weeks

III. Health events

The occurrence of respiratory diseases (runny nose, coughing etc.) and other types of disorders (umbilical infection) was not significantly different between the two groups (36% of calves 'Control' affected *vs.* 43% of calves 'Mother' affected, P=0.69 for respiratory diseases and 21% of calves affected in both groups for other types of disorders, P=1.00).

4.2 Behavioural observations results

4.2.1 Mother-young bond: *Effects of breed in mother-young relationship*

With the aim of evaluating the formation of the mother-young bond, we have considered the data collected at "Morning Reunion" observation and "During the Day" observation.

Table 4 *Behaviours related to calves-cows' interactions; adjusted means (±SE) values by breed Holstein (Ho and Montbéliarde (Mo).*

		Ho (n=7)	Mo (n=7)	Breed
Reunion (1h)	**Reunion latency (s)**	270.87 ± 65.52	232.15 ± 59.69	ns
	Refused suck attempts from Mothers	0	0	ns
	Refused suck attempts from Other cows	1.13 ± 0.23	0.80 ± 0.21	ns
	Successful suck attempts Tot	4.60 ± 0.66	6.74 ± 0.62	**
	Successful suck attempts from Mothers	2.11 ± 0.38	2.44 ± 0.36	ns
	Successful suck attempts from Other cows	2.65 ± 0.48	4.48 ± 0.44	**
During the Day (20min)	**Time spent interacting with Mother (s)**	1.20 ± 1.84	5.17 ± 1.84	ns
	Time spent interacting with Other cows (s)	2.51 ± 3.11	8.77 ± 3.11	ns

*(G)LMM : ***P<0.001; ** P<0.01; * P<0.05; + P<0.10; ns P≥0.10*

I. Calves-cows' interactions at "Morning Reunion" and "During the Day"

The *Reunion latency* time was 4 min, in average, and it was not significantly different between Mo and Ho calves.

No *Refused suck attempts from Mothers* were collected and *Refused request from Other cows* was about 1 attempt refused in average during 1h reunion. It was not different between the two breeds (P>0.10; *Table 4*).

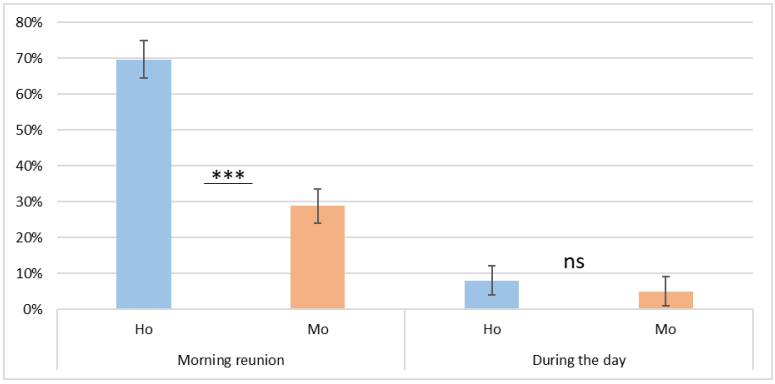
The *Total number of successful suck attempts* was 5.7 in average, corresponding to 2.3 attempts from the mother and 3.4 attempts from other cows. Ho calves have recorded less total successful suck attempts than Mo calves. This significant difference is due to the attempts from other cows. On the contrary, the successful suck attempts from mother is the same in the two breeds *(Table 4).*

The '*During the day'* observations evidence that the *Time spent interacting with the Mother* is lower in average than the *Time spent interacting with Other cows* (3.2 min and 5.6 min respectively). No

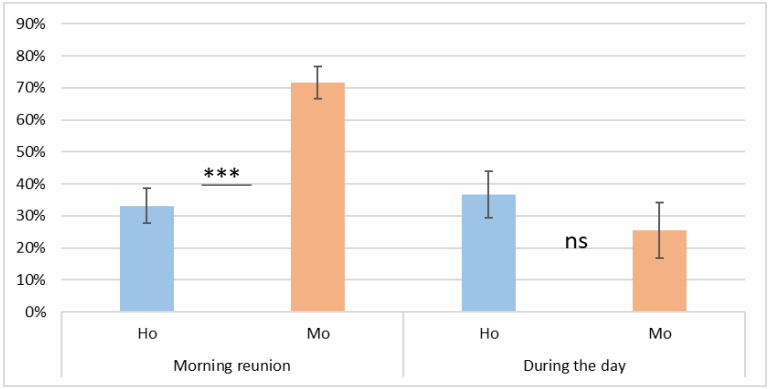
significant difference between breeds was observed but in average Ho calves spent less time interacting with their mother or other cows than did Mo calves (*Table 4*).

II. Effects of calves' breed on time spent suckling

During the morning reunion, calves spent in average half of their time suckling their mother and half suckling other cows. A significant difference between breeds (P<0.001) was observed: Ho calves spent more time suckling their mother than did Mo calves (respectively 70% *vs.* 30%) (*Fig. 15*) and on the contrary Mo calves spent more time suckling other cows (respectively 72% *vs.* 33%) (*Fig. 16*). Nevertheless, during the day we found no significant difference between breeds for both time spent suckling their mother or time spent suckling other cows.

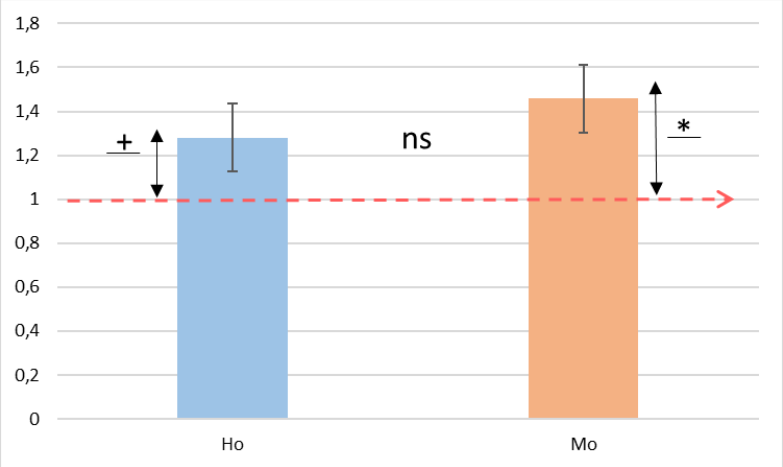


*(Fig. 15) Percentage of Time spent suckling mother at morning reunion and during the day according breeds. LMM, ***P<0.001; ** P<0.01; * P<0.05; + P<0.10; ns P≥0.10*



*(Fig. 16) Percentage of time spent suckling other cows (±SE) at morning reunion and during the day according breeds. LMM, ***P<0.001; ** P<0.01; * P<0.05; + P<0.10; ns P≥0.10*

III. Effects of cows' breed on selectivity



*(Fig. 17): Mean (±SE) of selectivity index of Holstein (Ho) and Montbéliarde (Mo). Tired line= value of no selectivity. LMr for breed comparison and Wilconxon T Test for comparison to value of no selectivity. ***P<0.001; ** P<0.01; * P<0.05; + P<0.10; ns P≥0.10*

The overall selectivity index of cows was not significantly different between breeds. However, the selectivity index was significantly above 1 for both breeds: they preferentially selected their own calf. This general tendency is significant for Mo (P<0.05) while it is not for Ho cows *(Fig. 17)*

4.2.2 Behaviour of calves during the day: *Effects of cow's presence on the behaviour of young calves*

I. Activities during the day according groups and breeds

Table 5 *Means (±SE) and statistical results (LMMr) of main behaviours which represent the activity of calves during 20 min of FOCUS according groups and breeds.*

	Calves Mother		Calves Control		*P*		
	Ho (n=7)	**Mo (n=7)**	**Ho (n=7)**	**Mo (n=7)**	**Group**	**Breed**	**Group*Breed**
General Activity (%)	0.16 ± 0.04	0.25 ± 0.04	0.18 ± 0.04	0.23 ± 0.05	ns	ns	ns
Suckling (%)	0.17 ± 0.02	0.07 ± 0.01	0.01 ± 0.02	0.02 ± 0.02	***	ns	ns
Feeding (%)	0.02 ± 0.02	0.07 ± 0.02	0.07 ± 0.02	0.08 ± 0.03	ns	ns	ns
Exploration (%)	0.05 ± 0.02	0.08 ± 0.02	0.09 ± 0.02	0.11 ± 0.03	ns	ns	ns
Laying (%)	0.80 ± 0.05	0.66 ± 0.05	0.71 ± 0.05	0.65 ± 0.06	ns	*	ns

****P<0,001; ** P<0,01; * P<0,05; + P<0,10; ns P≥0,10*

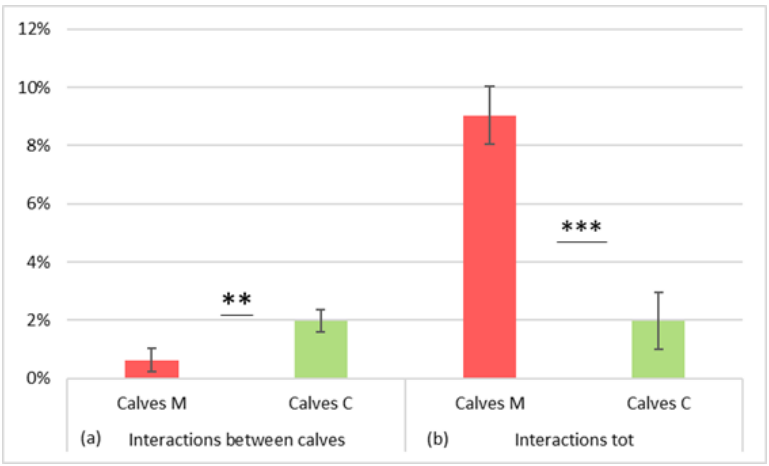
In average, the calves spent 71% of their time laying, 9% of their time exploring, 12% of their time feeding and 13% of their time suckling *(Table 5*).

The general activity of calves was not affected by the group or the breed and we found no interaction between group and breed. Nevertheless, the *Time spent Suckling* was significantly higher

(P<0.001) for calves 'Mother' than for calves 'Control' and *Time spent Laying* was significantly higher for calves Ho than calves Mo (P<0.05). We found no significant group*breed interaction.

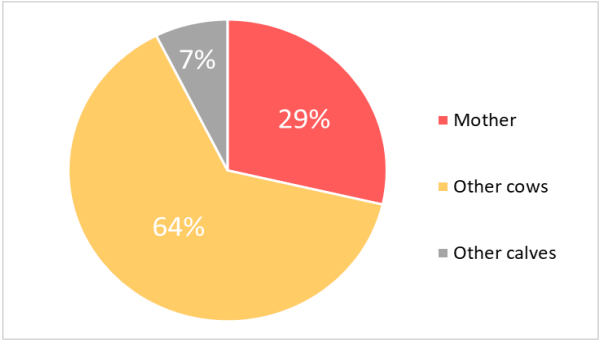
II. Analysis of calves' social interactions

At first, we have analyzed the *Positive social interactions between Calves* and we found a significant difference between the groups (P<0.01; *Fig. 18*): calves 'Mother' interacted less with other calves compared to calves 'Control'. Because of the presence of adult animals in group 'Mother', we have studied the *Total positive social interactions* (i.e. cows and calves for group 'Mother' and calves for group 'Control'): calves 'Mother' interacted significantly more with other individuals than calves 'Control' (P<0.001; *Fig. 18*). There was no effect of breed or group*breed interaction on time spent in positive interactions.



*(Fig. 18) Percentage of time spend in positive interactions with calves (a) and with all individuals (b) according groups. LMM, ***P<0,.001; ** P<0.01; * P<0.05; + P<0.10; ns P≥0.10*

As shown in *Figure 19*, calves 'Mother' spent more time interacting with adult animals than with coetaneous: they spent 64% of time with other cows, 29% of time with their mother and only 7% of time with other calves.



(Fig. 19) Distribution of total time spent in positive interactions in calves 'Mother'

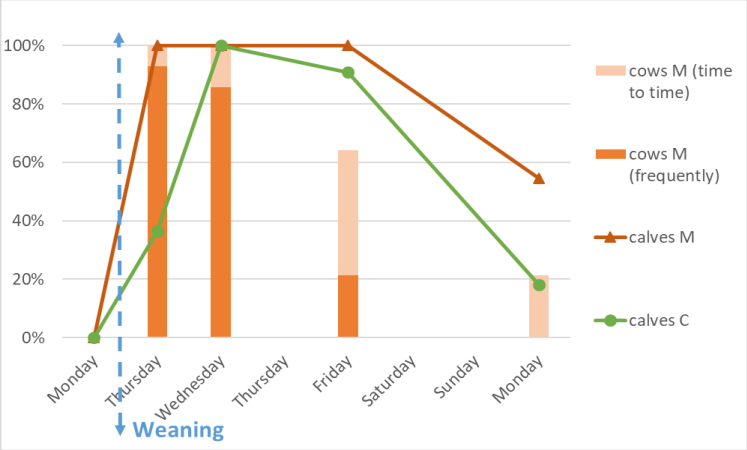
As regard calves' negative social interactions, no data were collected about calves' 'Mother' group. Calves 'Control' spent 33% ± 0,06 of the time in negative interactions with other individuals during the 20-minute FOCUS.

4.2.3 Weaning distress

I. Percentage of animals mooing during the week after weaning

As shown in *Figure 20*, immediately after weaning and for 4 days, 100% of calves 'Mother' reacted by mooing. After 1 week, 43% of calves 'Mother' still mooed. Calves 'Control' reached 100% of mooing with 1 day of delay and their reaction to weaning decreased faster (21% of mooing after 1 week).

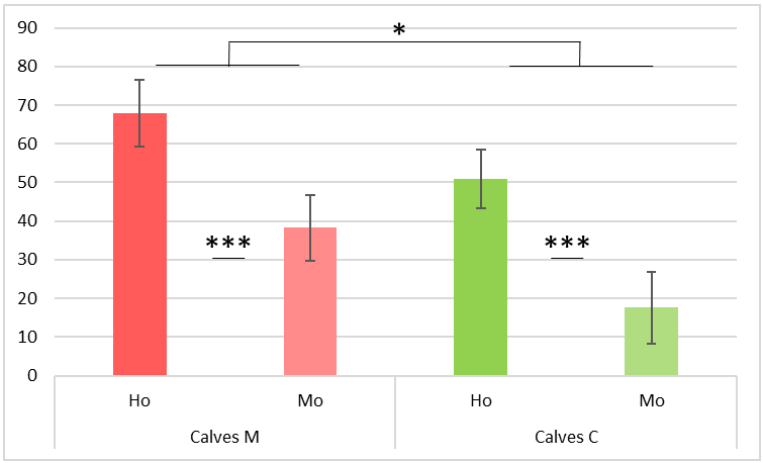
After weaning the reaction of the cows 'Mother' was similar to that of their calves (90% of cows mooed frequently and intensely), but it decreased more rapidly (21% of cows mooing time to time after 1 weeks).



(Fig. 20) Proportion of animals mooing during the week after weaning

II. Number of animals' vocalisations during the week after weaning

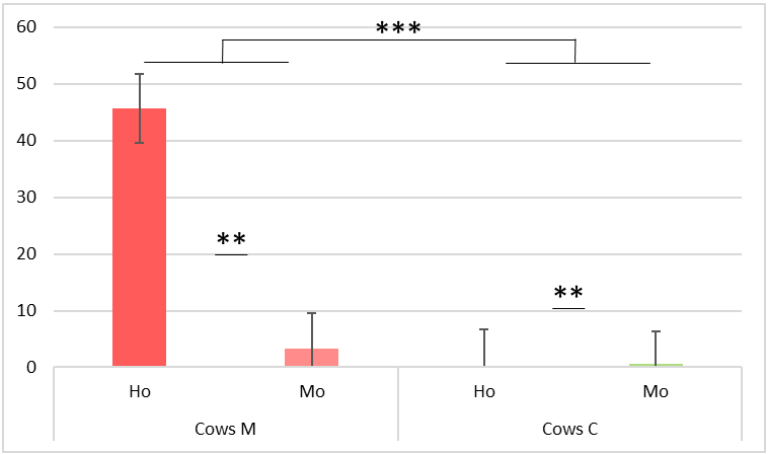
- Calves: the number of vocalizations was significantly different between the two groups ($P<0.05$): calves 'Mother' mooed more than calves 'Control' (respectively 53 voc./30 min *vs*. 34 voc./30 min; adjusted data). As regard the comparison of the two breeds within group, we found that calves Ho showed a higher number of vocalizations than the calves Mo ($P<0.001$). There was no effect of the group*breed interaction. (*Fig. 21*).



(Fig. 21) Total number of calves' vocalisation during the week after weaning

- Cows: the number of vocalizations was significantly different between the two groups (P<0.001): cows 'Mother' mooed more than cows 'Control' (respectively 25 voc./30 min *vs.* 0.4/30 min; adjusted data).

As regard the comparison of the two breeds within group, we found a significant difference between breeds (P=0.001): Ho cows showed a higher number of vocalisations than cows Mo (respectively 46% *vs.* 3%; adjusted data). (*Fig. 22*).



(Fig. 22) Total number of cows' vocalisation during the week after weaning

5. DISCUSSION

5.1 Animals performances

The main aim of our study was to investigate the effects of suckling system on milk production and on productive performances of cows and calves. In literature the effects of the natural suckling system were studied mainly from the point of view of calves and cows' welfare. Few authors have studied the effects of this practice on production performances of cows and calves (milk production, growth of calves, etc.).

> Effects of the suckling system on milk production and composition:

In our study we observed that the presence of the calf strongly reduces milk production. On average, we recorded a loss of 11 kg/d during the whole suckling period, which represents about 800 kg of milk per cow. Weary & Chua (2000) and Pomiès et al. (2010) also observed an important loss in milk production. The main cause of this loss is related to the quantity of milk ingested by the calves. In this study, it was not possible to measure the milk ingested by the calves and therefore the total milk production of the cows, but we can hypothesise that the calves of the 2 groups ingested almost the same amount of milk as their growth was similar. As the 'Control' calves ingested about 8 kg/d, the net loss of milk would be about 3 kg/d for cows 'Mother'. As expected, the loss of milk at evening milking during the first 8 weeks of lactation, (4.2 kg/d for cows 'Mother' *vs.* 10.7 kg/d for cows 'Control; raw data) was due to the milk consumed by calves during the day. Another hypothesis may be that the cows being frequently suckled, they had many releases of oxytocin during the day and therefore at the milking parlor they were no longer able to eject the milk (Lupoli et al., 2001). As regard the milk collected at morning milking during the first 8 weeks of lactation, we observed however an important loss (11.5 kg/d for cows 'Mother' *vs*. 16.1 kg/d for cows 'Control'; raw data). In fact, cows were separated from calves during the night (about 15h) so it was expected that the quantity of milk collected at the morning milking was not strongly influenced. We hypothesize that cows retained milk for calves for next suckling as suggested by Efsa et al. (2009).

In our study we found that the milk losses were higher for Holstein then for Montbéliarde cows (respectively -14.4 kg/d for Holstein *vs.* -8.2 kg/d for Montbéliarde; raw data). On the contrary other studies on restricted suckling systems highlighted that the Holstein cows, as selected for milk production, have less difficulty in ejecting milk than less selected breeds (Efsa et al., 2009). But from the analysis of behavioural data we found that the Holstein cows were more suckled than the Montbéliarde cows, and this could explain the higher loss of Holstein cows.

As expected, we found a lower fat content in machine-milked milk for cows of group 'Mother' than for cows of group 'Control'.

This could be due to the fact that cows 'Mother' retained milk for calves and consequently the fat content was not released to the milking machine, in particular at the morning milking. Anyway, compared to the evening milking, the milk fat content at morning milking was lower for both groups, because usually at morning the quantity of milk is higher and so the fat content is diluted (28.4 g/kg 'Mother' *vs.* 35.7 g/kg 'Control'). At evening milking the fat content of 'Mother' cows was higher than in the morning milking but still lower than in 'Control' cows (respectively 34.8 g/kg *vs*. 45.9 g/kg). This difference between the two groups is related to the removal of residual milk by the calves, which is the part of the milk with the highest fat content (Fröberg et al., 2007).

Concerning milk protein content, we found a new result: cows 'Mother' had a higher milk protein content production than cows 'Control'. The milk protein content is mainly related to the energy balance of the cow; the better the energy balance is, the higher the protein content is (Pomiès et al., 2010). We have not measured the feed ingestion but both groups received the same feed ration. So, we can only assume that the cows 'Mother' having received the same feed ration but having a lower milk production than cows 'Control', they have recorded a higher energy balance compared to the latter. Nevertheless, the results concerning BCS have not confirmed this hypothesis: BCS of cows 'Mother' was not higher than that of cows 'Control'. So further studies, including cow's ingestion measurements and plasma indicators of energy balance are necessary to interpret this result.

After the weaning of all calves, milk production and composition of both groups showed a similar trend (Metz et al., 1987). This shows that the disposition of the two groups on the basis of the productive potential of cows was balanced and that, therefore, the differences in performances observed are essentially due to the effect of the suckling system.

Suckling rearing system did not significantly affect the health of cows between the two groups. We found only a tendency of cows 'Mother' to have less health problems or events, but the number of animals taken into consideration is not sufficient to confirm it. We can hypothesize that according to the literature, suckling could decrease the risk of mastitis during the suckling period (Krohn, 2001). For mastitis, the slightly higher numerical milk SCC of cows 'Mother' seems contradictory with this hypothesis but this higher SCC count be linked to a concentration effect as the total production of cows seem to be reduced.

> Effects of suckling system on calves' growth:

As regards calves' performances, we found no difference between the two groups. In contrast to other studies (Flower & Weary, 2001; Johnsen, de Passille, et al., 2015) in which the suckling calves had a higher weight gain than artificial rearing calves, in our study the growth was similar for both groups. According to Metz et al. (1987) who affirms that a higher milk consumption led to a higher weight gain, we can hypothesise that calves 'Mother' ingested more or less the same quantities of milk as calves 'Control' (8.5 l/d). However, in the 3 weeks before weaning, we found that the calves 'Mother' grow faster and had a higher weight gain than calves 'Control'. This is consistent with Krohn et al. (2001) who shown that the faster growth is first due to a higher milk consumption but also to the social interaction between cow and calf which could give a positive effect on the daily gain of the calf.

Between the two groups we found no differences regarding the incidence of health events, but as for cows, the number of animals involved in this study was too limited to draw any conclusion on this specific question.

5.2 Weaning distress

>Effects of suckling system on weaning

The moment of weaning is the main critical point of the natural suckling system. The change of diet and the separation from the mother have a strong physical and psychological impact on both cows and calves. In our study, despite the separation of the animals for 15 hours a day, weaning had however a strong impact. From the point of view of production performances, we observed that after weaning cows had a decrease in production accompanied by a behavioural change that lasted for few days after weaning. Metz et al. (1987) found the same result: a 10-day contact between mother and its calf is enough to cause a decrease in production up to 5 days after separation. The decrease in production is a clear sign of distress and shows how the animal responds negatively to the separation. Similarly, for calves we recorded a weight loss during the first week after weaning. But, unlike cows, the calves of both groups have lost weight after weaning and this was due also to the elimination of milk from the diet that consequently requires time to animals to get used to a diet consisting only in solid feed. Therefore, it is important to highlight that, from the point of view of animal welfare at the time of separation, the suckling rearing system affects more strongly the welfare and the production of cows than that of calves.

It is evident that the weaning response affected only the cows 'Mother' and not the cows 'Control'. As shown by Veissier et al. (2013), before weaning, cows of both groups showed the same behaviour; but at weaning cows 'Mother' showed a marked change in behaviour, in terms of agitation and number of vocalizations. This pronounced behavioural response is an evident welfare problem and, as shown by our results, this stress period involved 100% of cows in the first two days after separation and lasts up to 4-5 days later with a decreasing percentage of animals involved.

As regards calves, weaning implied the separation from the mother for calves highly dependent on milk, while simultaneous being moved to a new environment (Fröberg et al., 2011). This shown the highest stress levels of calves 'Mother' compared to calves 'Control' during the first week after weaning. It is important to underline, however, that the vocalizations of the calves 'Control' have reached the peak 24 hours after weaning because, as described by (Flower & Weary, 2001), the response to weaning is delayed as it is related to the moment when they start to feel hunger. In fact, the calves 'Mother' were separated from the mother before the daily suckling while the calves control had available the 24h24 automatic milk dispenser and at time of weaning they could not be hungry. This late peak in vocalization is therefore due to a response to hunger, whereas the earlier calls may rather be an effect of social separation.

The study of the effect of the breed on weaning stress has shown that Holstein animals, both calves and cows, have responded to the separation with higher levels of vocalizations than the Montbéliarde ones. We hypothesize that this effect is due to a great emotionality of the Holstein race, even if we have not found evidence in the literature.

So, we can conclude that from the point of view of welfare, the suckling rearing system affects the behaviour of the cows more strongly than the behaviour of the calves. In fact, these latter are affected by weaning distress even in a classic rearing system.

5.3 Animal behaviour

>Effects of cow's presence on the behaviour of young calves

The presence of cows influenced the general activity of the two group of calves (Mother and Control) only about the time spent suckling during the day: we observed calves 'Mother' suckle more frequently during the day than calves 'Control'. As reported in other studies, in restricted suckling systems calves reared with their mother were observed suckling their mother more often than the artificially fed calves are seen at the milk feeder (Fröberg & Lidfors, 2009; Veissier et al., 2013). A possible explication of this result could be that the calves 'Mother' had a limited time during

the day to suckle. In fact, the time allowed to the calves in contact with the cows was 9h per day (from 7:30 am to 16:30 pm) while calves 'Control' had a possible access to the automatic milk dispenser 24 hours a day. Another hypothesis could be that the presence of cows stimulates more calves' motivation to suckle than the automatic milk dispenser. Roth et al. (2009) shown that milk intake via artificial teat does not fully satisfy the calves' motivation to perform suckling behaviour. In fact, for calves 'Mother', the frequency and the duration of suckling during the day was lower than the frequency and the duration of suckling at the first reunion in the morning: this can show that the animals were not motivated only by hunger but also by the need to satisfy a natural behaviour.

Regarding general activities, in particular *feeding* and *exploration*, we did not observe differences between the two groups. So, in our study the presence of cows doesn't seem to affect the general behaviour of the calves (*self-grooming, rumination, vocalization, locomotor play*). Wagner et al. (2015), on the contrary, found that calves with permanent access to their mothers and cows herd shown higher activity than classic rearing calves.

Nevertheless, the presence of cows influenced calves' social interactions. We found that calves reared with their mother showed more social interactions than artificially reared calves, as reported by Flower and Weary (2001). In particular they showed more interactions with adult individuals than coetaneous.

Interaction with adults has positive influences on the social behaviour of young animals: the development of a normal behaviour is better in calves reared with their dam than artificially reared calves. Contact with the mother and with other adult animals during rearing give to calves more opportunities for social experiences (Wagner et al., 2013). Adults impose social models, rules and herd's hierarchy from the early age and this is positively reflected on the social interactions of calves when they become adults (Bøe & Færevik, 2003). Furthermore, the maintenance of close proximity between mother and young provides opportunities for social transmission of information, such as information about feed sources or awareness of dangers (Newberry & Swanson, 2008).

To confirm this, we did not find any negative social interactions in calves 'Mother' calves reared with their mothers did not show aggressive or dominant behaviours (i.e. *pushing, kicking, head-butt*) in contrast to artificially reared calves. This is in opposition to Roth et al. (2008) results: they found that calves reared with the mother were threatening and fighting more often than artificially reared calves. However, in the study of Roth (2008), mother-reared calves were heavier than calves reared without mother due to a restricted milk allowance in the latter which was not the case in our study.

Wagner et al, instead, did not find any difference regarding the aggressive behaviour between calves reared with their dam and artificially reared calves.

> Effects of breed in mother-young in animal behaviour relationship

The mother-young bond was strong, even if the data collected were not analysed in this document. In fact, it was observed that during the day the calves spent most of their time in contact with their mother, in particular during the grazing period. Starting from this, in the next project this aspect will be studied more in depth.

The influence of the breed on the general activity of calves was low except for the time spent lying: we observed that the calves Ho spent more time in the position of decubitus compared to the calves Mo. Wagner et al. (2013) found that calves Black-and-White German Holstein were more active than German Red Pied and concluded that is a genetic effect: similarly Montbéliarde calves could be genetically more active than Holstein calves but this specific point is not documented in the literature.

The breed did not affect the mother-young relationship but substantial differences between breeds were found in the activity of calves, in particular during the first morning reunion where a breed effect in the choice of the cow to suckle was shown. In particular, we found that calves Mo tended to prefer other cows compared to their mother. As shown by Johnsen et al. (2015), the main motivation of calves at the first reunion is hunger and so they are highly motived to access milk promptly. For this reason, we hypothesize that the choice to prefer other cows compared to own mother derived simply from the search for easier access to milk. Indeed, Ho cows are known to produce higher amounts of milk and milk ejection is very rapid. Not only the availability of milk, but also the teats morphology and an easier milking affects the choice of veal motivated by hunger (Le Neindre, 1989). Calves Mo have recorded a greater number of successful suck attempts than the calves Ho, and specifically from other cows. This is in line with the fact that if they did not suckle their mother they were forced to change continually the cow to suckle. In fact, calves that suckle other cows usually take the behind or the anti-parallel position. These positions do not allow the cow to identify the calf by smelling or licking and, in addition, they suckle simultaneously with cow's own calf as a strategy to reduce the probability of rejection or aggression from the cow (Fröberg & Lidfors, 2009).

On the contrary, when a calf suckles its mother, it tends to spend more time to suckle because it is not rejected. In confirmation of this, the results showed that at the morning reunion 70% of calves Ho suckled their mother against 30% of calves Mo (*Fig. 23*).

(Fig. 23) Holstein cow suckled by its calf and other two calves Montbéilarde

Despite this, the latency time at the reunion with the mother was not different between the two breeds. On average each calf came into contact with his mother after 4 min from the access to the collective park. A positive result confirming the formation of a mother-young bond is the total absence of refused suck attempts from mothers towards their calves. As regards the refused suck attempts from other cows, we found no differences between the two breeds, although it seemed that the calves Ho received more rejects from the other cows. This can lead to the hypothesis that the cows Mo are more selective. The analysis of the selectivity has in fact shown that both breeds are selective towards their calf, but more particularly the Mo cows. This refutes the idea that cows Mo did not have maternal behaviour, since their calves suckled other cows. Le Neindre et al. (1989) studied the maternal behaviour of Holstein cows compared to Salers cows: they found that Holstein cows were less selective towards alien calves than Salers cows, and they concluded that low calf selectivity is linked to easy milking, and the milking ability of Holstein is higher than that of Salers. They also found that mother-young contact is more important for calves Salers than for Holstein calves, and this is due to a breed effect and to the distinctive rearing system (the two types of animals appears to be adapted to specific environments). The total suckling time should result from the need for mother-young physical contact instead of milk production.

We found no differences for mother-young bond during the day: the time spent interacting with mother and other cows was similar for both calves' breeds. Although not significant, the calves Ho

seemed to tend to suckle other cows during the day. A possible motivation may be that the mothers of Ho calves had less milk available during the day because they shared most of their milk with alien calves at the first morning reunion. Furthermore, it can be noted that the calves Mo tend to suckle their mothers during the day, this could confirm that cows Mo had more milk available during the day, considering that they had not been suckled at the first reunion in the morning.

Finally, it is important to specify that the results obtained from the analysis of behavioral data are the result of a preliminary analysis. The reason is the insufficient time available to study all the data collected with the cameras and elaborate more in deep the data collected by the observers. So probably the effect of the suckling system on the behavior of the animals can be analyzed more in depth. The real results will be analyzed in the coming years and will end with a scientific publication.

5.4 Limits of this study

As regards the methods of execution of our study, in light of the results obtained, we have realized that the weaning technique used could be improved. In fact, it has strongly influenced the welfare and production of cows. The criticism concerns to the fact that at the time of weaning the cow was not moved to another park, as its calf. This choice negatively influenced the behaviour of cows because, not changing the environment, they expected to find their calf during the day and the inability to find it caused a strong level of stress. At the same time, despite the absence of their calves, they still remained in contact with the calves not yet weaned and this influenced the production, as they could still be suckled. As shown by the results in fact, only at the weaning of the last calf the average production of the cows 'Mother' has reached the average production of the cows 'Control'. In conclusion we can affirm that to reduce the effect of the separation on the production and on the behaviour of the cows, the best choice is to remove from the collective park not only the calves but also the corresponding mothers. This modality could therefore partly reduce the stress of the cows as it could speed up the time in which they forget their calves and, above all, prevent the loss of milk due to the suckling of the other calves.

6. CONCLUSIONS

The results obtained in this study evidenced that the natural suckling system affects both the production performance and the behaviour and welfare of the animals. The presence of positive and at the same time negative aspects does not allow us to give a unidirectional opinion on the evaluation of this system.

This system has a strong impact on milk production in terms of quantity and composition. Loss of milk is substantial compared to a classic rearing system. The reduction in milk fat content and, in our case, the increase in milk protein content affects the composition and quality of the milk. On the other hand, the performances of the calves were positively affected by this practice.

The system had positive effects on animal behaviour, because it allows animals to express a behavioural repertoire more similar to natural conditions. This system has influenced the maternal behaviour of the cows in terms of preferences towards their calves and has influenced the social interactions of the calves 'Mother': the presence of cows stimulates the social interaction of young animals. Although it has not been expressly studied in our work, the benefit of this practice on the calves' welfare is the reduction, and sometimes the total absence (Roth et al., 2009), of abnormal behaviour such as cross-suckling. And this proof the absence of frustration and distress in animals. This is a crucial aspect especially as regards the opinion of consumers, which becomes increasingly attentive and critical to current farming practices.

For the farmer this practice could be positive in terms of working time reduction: leaving the calves fed by the mothers implies the elimination of the working time employed in the management of calves feeding control (natural suckling system may save labour by allowing the cows to take care of their offspring) (Fröberg et al., 2011). The separation of animals at the evening milking did not show to be (has not been shown) an additional work: when the farmer leads the cows to the milking parlor for the evening milking he only has to separate the calves from the mother and place them in their park aside.

The more critical aspect of this practice is the moment of weaning. It is important to remember that weaning is always a stressful moment for calves, even in the classic breeding system (Weary & Chua, 2000). But in suckling rearing system, the impact on animal welfare is even strongest, and above all it involves both calves and cows. The separation of the animals causes a strong stress that lasts for a few days and affects the production of milk for the cows and the growth of the calves. But the

behavioural response at separation should be weighed against the benefits of cow–calf suckling as compared to artificial rearing where the calf is separated from the dam shortly after birth. Suckling calves can improve their weight gains (Flower & Weary, 2001), may be healthier (Weary & Chua, 2000) and show natural behaviours (Johnsen et al., 2015) and dairy cows suckling have the possibility to express maternal behaviour. Delayed separation provides advantages also in term of social behaviour, so all these factors can compensate the increase behavioural response to separation.

In conclusion, further studies are needed to find the best solution to guarantee both animal welfare and satisfactory production level. Moreover it is still necessary to work to define the optimal weaning method, because the late separation remains the critical point of this practice.

7. ACKNOWLEDGEMENTS

Innanzitutto vorrei ringraziare i miei supervisori Enrico Sturaro, Bruno Martin e Dominique Pomiès per avermi dato la possibilità di poter prendere parte a questo progetto di tesi. Li ringrazio per la fiducia e la stima che mi hanno dato in questi mesi. In particolare ringrazio Bruno e Dominique per la gentilezza che hanno avuto nei miei confronti durante il mio periodo in Francia, per essere stati disponibili nelle difficoltà e per avermi accolta non come una semplice stagista ma come quasi una figlia. Ringrazio il mio prof. Enrico Sturaro che nonostante la distanza è sempre stato presente e che grazie a lui ora ho la possibilità di continuare il mio percorso formativo. Ringrazio inoltre Nadege Aigueperse per l'aiuto e la pazienza che mi ha dato nella raccolta ed elaborazione dei dati comportamentali.

Ringrazio di cuore tutte le persone che hanno lavorato con me in azienda a Marcenat. Li ringrazio perché mi hanno accolta a braccia aperte e mi hanno accompagnata giorno per giorno sostenendomi in ogni piccola difficoltà. Grazie a loro non mi sono mai sentita sola. In particolare ringrazio Monique per la sua infinita dolcezza e gentilezza che fin dal primo giorno si è preoccupata per me e mi ha regalato la sua disponibilità in qualsiasi occasione. Ringrazio Olivier per avermi fatto da taxista praticamente tutti i giorni e per aver cercato in tutti i modi di sistemarmi i termosifoni di casa (e mi scuso per non avergli mai cucinato una pizza italiana). Ringrazio Florence e Matthieu per la loro simpatia e disponibilità e per avermi dato piena fiducia e piena responsabilità nel seguire e gestire gli animali della mia prova in tutti i suoi aspetti. Ringrazio Cyril, Eric, Francois, Bob, Jérôme e tutti gli altri animaliers per avermi insegnato a lavorare con gli animali e soprattutto per aver avuto la pazienza di affiancarmi e di seguirmi in tutte le mie innumerevoli osservazioni comportamentali (e per tutti gli aperitivi a chez Simone). Ringrazio Veronique per il suo cibo che, anche se un po' bizzarro per un'italiana, ho sempre apprezzato per l'amore che ci metteva nel prepararlo (e soprattutto la ringrazio per avermi offerto tutti i giorni gli avanzi del pranzo evitandomi così di cucinare la sera). Ringrazio Carole per la sua simpatia e per l'aiuto che mi ha dato in tutti i problemi tecnologici e di gestione dei dati che ho incontrato perché non so come avrei fatto senza di lei. Ringrazio Renan per la sua logorrea e il suo modo di parlare strano che non capivo quasi mai niente di quello che mi diceva e a lui comunque non importava, e lo ringrazio soprattutto per la sua disponibilità e gentilezza che ha sempre avuto nei miei confronti. Ringrazio Philippe Pradel, Philippe Tardif (Fifi), André (Dede) e tutti gli altri perché ognuno di loro, almeno con un gesto, mi ha fatto sentire accolta e apprezzata. Infine Lise, che da quando è arrivata a Marcenat ha cambiato la mia vita. Non la ringrazierò mai abbastanza per tutte le sue gentilezze, dall'accompagnarmi ogni giorno

a lavoro all'ospitarmi a casa sua e a farmi sentire parte della sua famiglia. La ringrazio per le risate, per gli aperitivi chez Simone, per avermi fatto visitare il Cantal e per tutta la sua infinità bontà.
Ringrazio Marcenat per le difficoltà che mi ha fatto affrontare e soprattutto superare perché mi hanno resa più grande e più forte. Ringrazio la neve, il freddo, la solitudine ma anche le montagne, i paesaggi mozzafiato e le persone che ho incontrato, che anche se per pochi minuti, mai mi hanno fatta sentire straniera.
Ringrazio tutte le persone che ho incontrato all'INRA a Theix. A partire dai miei colleghi di ufficio, Pauline, Thaïs, Adeline e Nicola che hanno reso tutti i giorni sempre un po' caotici ma divertenti e ricchi di bei momenti. Ringrazio tutti i ragazzi del gruppo «Non Titulaires», Louise, Cécile, Eglantine, Francois, Ikram, Maeva, Marie, Nicolas, Anderson e Ruben per tutti i pranzi, tutti gli aperitivi al barbar e per tutte le serate di festa passate insieme. Ma in particolare voglio ringraziare Hélène, la mia primissima compagna di avventure che mi ha affiancato dal primo fino all'ultimo giorno. A lei devo tutti i più bei momenti, le più belle risate e le più belle avventure, e so che in lei ho trovato un'amica vera e sincera che porterò avanti nella vita. Ringrazio Jimmy per avermi fatto conoscere la vera Clermont-Ferrand, per le gite, i concerti e i festival (e per aver portato le mie chiavi di casa in vacanza in Portogallo) ma soprattutto per avermi accolto nel suo gruppo di amici, Mélodie, Marie, Lucas, Quentin, Rémi e Greg che mi hanno accettata fin da subito e che mi hanno rinfacciato ogni santo giorno che l'Italia non ha giocato ai mondiali e che sono loro i campioni del mondo.
Ringrazio Nicola, a cui voglio un bene grandissimo. Lo ringrazio per aver vissuto con me questa fantastica avventura, che ci ha cambiati e migliorati in ogni aspetto. Lo ringrazio per avermi fatto praticamente sempre dannare, per tutti i bisticci che abbiamo avuto che in fin dei conti ci hanno portato a volerci sempre più bene, ma soprattutto lo ringrazio per il sostegno che ci siamo dati a vicenda in ogni momento. Senza di lui non so come avrei fatto.
Infine ringrazio la mia famiglia, perché ovviamente senza di loro non avrei potuto vivere tutto questo. Ringrazio la mia sorellina Francesca per essermi sempre stata accanto in questi mesi, soprattutto nei momenti di difficoltà. Ringrazio le mie amiche, che nonostante la distanza si sono sempre fatte sentire vicine, Angela, Silvia, Fra e Babu. In particolare Angela per le sue chiamate in cui mi ha sempre fatto ridere e ricordato di essere ancora italiana, e Silvia per esserci sostenute a vicenda nei momenti di solitudine a Marcenat per me e in Africa per lei.
È stata l'esperienza più bella della mia vita e rimarrà sempre nel mio cuore.

Je voudrais tout d'abord remercier mes superviseurs Enrico Sturaro, Bruno Martin et Dominique Pomiès de m'avoir donné l'occasion de participer à ce projet. Je les remercie pour la confiance et l'estime qu'ils m'ont données durant tous ces mois. En particulier, je remercie Bruno et Dominique pour la gentillesse qu'ils ont eue envers moi pendant mon séjour en France, pour avoir été disponibles en cas de difficultés et pour m'avoir accepté non pas comme simple stagiaire mais presque comme leur fille. Je remercie mon prof. Enrico Sturaro qui a toujours été présent malgré la distance et grâce à qui j'ai maintenant la possibilité de continuer ma formation. Je remercie également Nadege Aigueperse pour l'aide et la patience qu'elle m'a données dans la collecte et le traitement des données comportementales.

Je remercie sincèrement toutes les personnes qui ont travaillé avec moi à Marcenat. Je les remercie car ils m'ont accueilli à bras ouverts et ils m'ont accompagnée jour après jour pour me soutenir dans chaque petite difficulté. Grâce à eux, je ne me suis jamais senti seule. En particulier, je remercie Monique pour sa tendresse et sa gentillesse infinies et qui, dès le premier jour, s'est rendu disponible pour moi en toute occasion. Je remercie Olivier pour avoir été mon chauffeur pratiquement tous les jours et pour avoir essayé de toutes les manières d'ajuster les radiateurs chez moi (et je m'excuse de ne lui avoir jamais cuisiné une pizza italienne). Je remercie Florence et Matthieu pour leur sympathie et leur disponibilité et pour m'avoir donné leur pleine confiance et la pleine responsabilité du suivi des animaux de mon projet sous tous ses aspects. Je remercie Cyril, Éric, François, Bob, Jérôme et tous les autres animaliers pour m'avoir appris à travailler avec les animaux et surtout avoir eu la patience de me supporter et de me suivre dans mes innombrables observations comportementales (et aussi pour tous les apéritifs chez Simone). Je remercie Véronique pour les repas que j'ai toujours appréciés (bien que la nourriture ici soit un peu bizarre pour une Italienne) pour l'amour qu'elle mettait à les préparer (et surtout je la remercie de m'avoir donné les restes du déjeuner tous les jours, elle m'a évité de cuisiner chaque soir). Je remercie Carole pour sa sympathie et l'aide qu'elle m'a apportée dans tous les problèmes technologiques et de gestion de données que j'ai rencontrés car je ne sais pas comment je les aurais résolus sans elle. Je remercie Renan pour ses bavardages et sa drôle de façon de parler, qui m'a souvent été difficile à comprendre, ce qui l'amusait beaucoup et je le remercie aussi pour sa disponibilité et sa gentillesse. Je remercie Philippe Pradel, Philippe Tardif (Fifi), André (Dede) et tous les autres car chacun d'eux, au moins par un geste, m'a fait sentir bienvenue et appréciée. Enfin, Lise, qui a changé ma vie depuis son arrivée à Marcenat. Je ne la remercierai jamais assez pour toute sa gentillesse, pour m'avoir accompagnée au travail tous les jours et m'avoir fait sentir

comme un membre de sa famille. Je la remercie pour les rires, pour les apéritifs chez Simone, pour m'avoir fait visiter le Cantal et pour toute sa bonté infinie.
Je remercie le village de Marcenat pour les difficultés qu'il m'a fait vivre mais surtout surmontées car elles m'ont rendu plus grande et plus forte. Je remercie la neige, le froid, la solitude mais aussi les montagnes, les paysages à couper le souffle et les gens que j'ai rencontrés, même pour quelques minutes, et qui ne m'ont jamais fait sentir comme une étrangère.
Je remercie toutes les personnes que j'ai rencontrées à l'INRA de Theix. En commençant par mes collègues du bureau, Pauline, Thaïs, Adeline et Nicola, qui ont rendu chaque jour un peu chaotique mais amusant et riche de bons moments. Je remercie tous les «Non Titulaires», Louise, Cécile, Eglantine, François, Ikram, Maeva, Marie, Nicolas, Anderson et Ruben pour tous les déjeuners, tous les apéritifs au BarBar et pour toutes les soirées de fête ensemble. Mais en particulier, je tiens à remercier Hélène, ma toute première compagne d'aventures qui m'a accompagnée du premier au dernier jour. Je lui dois les meilleurs moments, les plus beaux rires et les plus belles aventures, et je sais qu'en elle j'ai trouvé une amie sincère que je porterai dans ma vie. Je remercie Jimmy de m'avoir fait connaitre le vrai Clermont-Ferrand, pour les concerts et les festivals (et pour avoir emmené les clés de chez moi en vacances au Portugal) mais surtout pour m'avoir accueilli dans son groupe d'amis, Mélodie, Marie, Lucas, Quentin, Rémi et Greg qui m'ont acceptée tout de suite et qui m'ont rappelé chaque jour que l'Italie ne jouait pas à la coupe du monde et que ce sont eux les champions du monde.

MERCI À TOUS!

8. BIBLIOGRAPHY

Agenäs, S. (2017). Editorial: We need to bring the calves back to the dairy cows. *Journal of Dairy Research*, *84*(3), 239. https://doi.org/10.1017/S0022029917000346

Bøe, K. E., & Færevik, G. (2003). Grouping and social preferences in calves, heifers and cows. *Applied Animal Behaviour Science*, *80*(3), 175–190. https://doi.org/10.1016/S0168-1591(02)00217-4

Busch, G., Weary, D. M., Spiller, A., & Von Keyserlingk, M. A. G. (2017). American and German attitudes towards cowcalf separation on dairy farms. *PLoS ONE*, *12*(3), 1–20. https://doi.org/10.1371/journal.pone.0174013

Council of the European Union. (2009). Council Directive 2008/119/EC of 18 December 2008 laying down minimum standards for the protection of calves. *Official Journal of the European Union*, *10/7*, 7–13.

de Passillé, A. M., Marnet, P.-G., Lapierre, H., & Rushen, J. (2008). Effects of Twice-Daily Nursing on Milk Ejection and Milk Yield During Nursing and Milking in Dairy Cows. *Journal of Dairy Science*, *91*(4), 1416–1422. https://doi.org/10.3168/jds.2007-0504

Edwards, S. A., & Broom, D. M. (1982). Behavioural interactions of dairy cows with their newborn calves and the effects of parity. *Animal Behaviour*, *30*(2), 525–535. https://doi.org/10.1016/S0003-3472(82)80065-1

Efsa, Q. N., Bertoni, G., Lidfors, L., Oltenacu, P., Rushen, J., & Stilwell, G. (2009). Scientific report on the effects of farming systems on dairy cow welfare and disease. *EFSA Journal*, *7*(7), 1143r. https://doi.org/10.2903/j.efsa.2009.1143r

Flower, F. C., & Weary, D. M. (2001). Effects of early separation on the dairy cow and calf: 2. Separation at 1 day and 2 weeks after birth. *Applied Animal Behaviour Science*, *70*(4), 275–284. https://doi.org/10.1016/S0168-1591(00)00164-7

Fröberg, S., Aspegren-Güldorff, A., Olsson, I., Marin, B., Berg, C., Hernández, C., … Svennersten-Sjaunja, K. (2007). Effect of restricted suckling on milk yield, milk composition and udder health in cows and behaviour and weight gain in calves, in dual-purpose cattle in the tropics. *Tropical Animal Health and Production*, *39*(1), 71–81. https://doi.org/10.1007/s11250-006-4418-0

Fröberg, S., & Lidfors, L. (2009). Behaviour of dairy calves suckling the dam in a barn with automatic

milking or being fed milk substitute from an automatic feeder in a group pen. *Applied Animal Behaviour Science*, *117*(3–4), 150–158. https://doi.org/10.1016/j.applanim.2008.12.015

Fröberg, S., Lidfors, L., Svennersten-Sjaunja, K., & Olsson, I. (2011). Performance of free suckling dairy calves in an automatic milking system and their behaviour at weaning. *Acta Agriculturae Scandinavica A: Animal Sciences*, *61*(3), 145–156. https://doi.org/10.1080/09064702.2011.632433

Grøndahl, A. M., Skancke, E. M., Mejdell, C. M., & Jansen, J. H. (2007). Growth rate, health and welfare in a dairy herd with natural suckling until 6-8 weeks of age: A case report. *Acta Veterinaria Scandinavica*, *49*(1), 1–6. https://doi.org/10.1186/1751-0147-49-16

Hale, S. A., Capuco, A. V., & Erdman, R. A. (2003). Milk Yield and Mammary Growth Effects Due to Increased Milking Frequency During Early Lactation. *Journal of Dairy Science*, *86*(6), 2061–2071. https://doi.org/10.3168/jds.S0022-0302(03)73795-3

Haley, D. B., Bailey, D. W., & Stookey, J. M. (2005). The effects of weaning beef calves in two stages on their behaviour and growth rate. *Journal of Animal Science*, (Stage 1), 2205–2214. https://doi.org/10.2527/2005.8392205x

Hudson, S. J., & Mullord, M. M. (1977). Investigations of maternal bonding in dairy cattle. *Applied Animal Ethology*, *3*(3), 271–276. https://doi.org/10.1016/0304-3762(77)90008-6

Johnsen, J. F., de Passille, A. M., Mejdell, C. M., Bøe, K. E., Grøndahl, A. M., Beaver, A., … Weary, D. M. (2015). The effect of nursing on the cow-calf bond. *Applied Animal Behaviour Science*, *163*, 50–57. https://doi.org/10.1016/j.applanim.2014.12.003

Johnsen, J. F., Ellingsen, K., Grøndahl, A. M., Bøe, K. E., Lidfors, L., & Mejdell, C. M. (2015). The effect of physical contact between dairy cows and calves during separation on their post-separation behavioural response. *Applied Animal Behaviour Science*, *166*(1), 11–19. https://doi.org/10.1016/j.applanim.2015.03.002

Johnsen, J. F., Zipp, K. A., Kälber, T., Passillé, A. M. de, Knierim, U., Barth, K., & Mejdell, C. M. (2016). Is rearing calves with the dam a feasible option for dairy farms?—Current and future research. *Applied Animal Behaviour Science*, *181*, 1–11. https://doi.org/10.1016/j.applanim.2015.11.011

Krohn, C. C. (2001). Effects of different suckling systems on milk production, udder health, reproduction, calf growth and some behavioural aspects in high producing dairy cows - A

review. *Applied Animal Behaviour Science*, *72*(3), 271–280. https://doi.org/10.1016/S0168-1591(01)00117-4

Le Cozler, Y., Recoursé, O., Ganche, E., Giraud, D., Danel, J., Bertin, M., & Brunschwig, P. (2012). A survey on dairy heifer farm management practices in a Western-European plainland, the French Pays de la Loire region. *Journal of Agricultural Science*, *150*(4), 518–533. https://doi.org/10.1017/S0021859612000032

Le Neindre, P. (1989). Influence of cattle rearing conditions and breed on social relationships of mother and young. *Applied Animal Behaviour Science*, *23*(1–2), 117–127. https://doi.org/10.1016/0168-1591(89)90012-9

Lidfors, L. M. (1996). Behavioural effects of separating the dairy calf immediately or 4 days post-partum. *Applied Animal Behaviour Science*, *49*(3), 269–283. https://doi.org/10.1016/0168-1591(96)01053-2

Loberg, J. M., Hernandez, C. E., Thierfelder, T., Jensen, M. B., Berg, C., & Lidfors, L. (2008). Weaning and separation in two steps-A way to decrease stress in dairy calves suckled by foster cows. *Applied Animal Behaviour Science*, *111*(3–4), 222–234. https://doi.org/10.1016/j.applanim.2007.06.011

Lupoli, B., Johansson, B., Uvnäs-Moberg, K., & Svennersten-Sjaunja, K. (2001). Effect of suckling on the release of oxytocin, prolactin, cortisol, gastrin, cholecystokinin, somatostatin and insulin in dairy cows and their calves. *Journal of Dairy Research*, *68*(2), 175–187. https://doi.org/10.1017/S0022029901004721

Mendoza, A., Cavestany, D., Roig, G., Ariztia, J., Pereira, C., La Manna, A., … Galina, C. S. (2010). Effect of restricted suckling on milk yield, composition and flow, udder health, and postpartum anoestrus in grazing Holstein cows. *Livestock Science*, *127*(1), 60–66. https://doi.org/10.1016/j.livsci.2009.08.006

Metz, J. (1987). Productivity aspects of keeping dairy cow and calf together in the post-partum period. *Livestock Production Science*, *16*(4), 385–394. https://doi.org/10.1016/0301-6226(87)90007-8

Newberry, R. C., & Swanson, J. C. (2008). Implications of breaking mother-young social bonds. *Applied Animal Behaviour Science*, *110*(1–2), 3–23.

https://doi.org/10.1016/j.applanim.2007.03.021

Pomiès, D., Bouchon, M., Veissier, I., & Martin, B. (2018). Suckling of dairy calves by their dams: consequences of a welfare-friendly pratice on milk yield, milk composition and growth of the calves. In *10th International Symposium in the Nutrition of Herbivores*. Clermont-Ferrand, France.

Pomiès, D., Caré, S., Veissier, I., Monts, U. E., Orcival, F.-, & Care, S. (2010). Once daily milking combined with suckling in Holstein cows, (3), 233–236.

Price, E. O., Harris, J. E., Borgwardt, R. E., Sween, M. L., & Connor, J. M. (2014). Fenceline contact of beef calves with their dams at weaning reduces the negative effects of separation on behavior and growth rate The online version of this article , along with updated information and services , is located on the World Wide Web at : Fen, (July), 116–121.

Roth, B. (2008). The effect of artificial Rearing on the develoment of suckling behaviour, performance and stress reactivity in dairy calves. https://doi.org/10.3929/ethz-a-005757149

Roth, B. A., Barth, K., Gygax, L., & Hillmann, E. (2009). Influence of artificial vs. mother-bonded rearing on sucking behaviour, health and weight gain in calves. *Applied Animal Behaviour Science*, *119*(3–4), 143–150. https://doi.org/10.1016/j.applanim.2009.03.004

Sandoval-Castro, C. A., Anderson, S., & Leaver, J. D. (2000). Production responses of tropical crossbred cattle to supplementary feeding and to different milking and restricted suckling regimes. *Livestock Production Science*, *66*(1), 13–23. https://doi.org/10.1016/S0301-6226(00)00164-0

Tesorero, M., Combellas, J., Uzcátegui, W., & Gabaldón, L. (2001). Influence of suckling before milking on yield and composition of milk from dual purpose cows with restricted suckling. *Livestock Research for Rural Development*.

The Couincil of the European Union, & of the, E. U. (2007). Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. *Official Journal of the European Union*, *L 189/1*(20), 1–23. https://doi.org/2004R0726 - v.7 of 05.06.2013

Uvnäs-Moberg, K., Johansson, B., Lupoli, B., & Svennersten-Sjaunja, K. (2001). Oxytocin facilitates behavioural, metabolic and physiological adaptations during lactation. *Applied Animal*

Behaviour Science, *72*(3), 225–234. https://doi.org/10.1016/S0168-1591(01)00112-5

Veissier, I., Caré, S., & Pomiès, D. (2013). Suckling, weaning, and the development of oral behaviours in dairy calves. *Applied Animal Behaviour Science*, *147*(1–2), 11–18. https://doi.org/10.1016/j.applanim.2013.05.002

Veissier, I., Lamy, D., & Le Neindre, P. (1990). Social behaviour in domestic beef cattle when yearling calves are left with the cows for the next calving. *Applied Animal Behaviour Science*, *27*(3), 193–200. https://doi.org/10.1016/0168-1591(90)90056-J

Ventura, B. A., von Keyserlingk, M. A. G., Schuppli, C. A., & Weary, D. M. (2013). Views on contentious practices in dairy farming: The case of early cow-calf separation. *Journal of Dairy Science*, *96*(9), 6105–6116. https://doi.org/10.3168/jds.2012-6040

von Keyserlingk, M. A. G., & Weary, D. M. (2007). Maternal behavior in cattle. *Hormones and Behavior*, *52*(1), 106–113. https://doi.org/10.1016/j.yhbeh.2007.03.015

Wagenaar, J. . (2009). Happy Dutch organic calves: suckling systems in organic dairying in the Netherlands. *Calf Welfare in Organic Herds - Planning for the Future*, 17–22.

Wagenaar, J. P. T. ., & Langhout, J. (2007). Suckling systems in calf rearing in organic dairy farming in the Netherlands. *QLIF Congress, Hohenheim, Germany*.

Wagner, K., Barth, K., Hillmann, E., Palme, R., Futschik, A., & Waiblinger, S. (2013). Mother rearing of dairy calves: Reactions to isolation and to confrontation with an unfamiliar conspecific in a new environment. *Applied Animal Behaviour Science*, *147*(1–2), 43–54. https://doi.org/10.1016/j.applanim.2013.04.010

Wagner, K., Seitner, D., Barth, K., Palme, R., Futschik, A., & Waiblinger, S. (2015). Effects of mother versus artificial rearing during the first 12 weeks of life on challenge responses of dairy cows. *Applied Animal Behaviour Science*, *164*, 1–11. https://doi.org/10.1016/j.applanim.2014.12.010

Weary, D. M., & Chua, B. (2000). Effects of early separation on the dairy cow and calf: 1. Separation at 6 h, 1 day and 4 days after birth. *Applied Animal Behaviour Science*, *69*(3), 177–188. https://doi.org/10.1016/S0168-1591(00)00128-3

Weary, D. M., Jasper, J., & Hötzel, M. J. (2008). Understanding weaning distress. *Applied Animal Behaviour Science*, *110*(1–2), 24–41. https://doi.org/10.1016/j.applanim.2007.03.025