Head Office: Università degli Studi di Padova

Department of

Agronomy, Food, Natural resources, Animals and Environment (DAFNAE)

Ph.D. COURSE IN: ANIMAL AND FOOD SCIENCE

SERIES XXXIV

SUCKLING OF DAIRY CALVES BY THEIR DAMS: CONSEQUENCES ON PERFORMANCE, FEEDING BEHAVIOR AND ANIMAL WELFARE

Thesis written with the financial contribution of Fondazione Cariparo

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Dipartimento di

Agronomia Animali Alimenti Risorse Naturali e Ambiente (DAFNAE)

CORSO DI DOTTORATO DI RICERCA IN: ANIMAL AND FOOD SCIENCE

CICLO XXXIV

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List of Abbreviations

Ad lib	Ad libitum
ADF	Acid Detergent Fiber
ADG	Average Daily Gain
AWU	Annual Work Unit
BCS	Body Condition Score
BHB	Beta-Hydroxybutyrate
BVD	Bovine Viral Diarrhea
BW	Body Weight
C	Control
CCC	Cow-Calf Contact
CP	Crude Protein
D	Dam
DM	Dry matter
FA	Fatty Acid
HAR	Human-animal relationship
Ho	Holstein
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LF	Lactoferrin
M	Mixed
Mo	Montbéliarde
NDF	Neutral Detergent Fiber
NEB	Negative Energy Balance
NEFA	Non-Esterified Fatty Acids
PDO	Protected Designation of Origin
PI	Persistently infected
S	Standard
SCC	Somatic Cell Count
UFL	Forage Unit for Lactation
Wk	Week

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Abstract

In most European dairy farms, calves are usually separated from their dams immediately or within few hours after birth. They are housed in individual pens for 1 or 2 weeks, then male calves are generally sold to be fattened in specialized farms and female calves, which are the future replacement heifers, are reared in multiple pens. In dairy systems, weaning corresponds to the suppression of milk in the diet and it generally occurs around 8-10 weeks of age. Early separation of the calf from the cow is a keystone of modern dairy systems. Consumers are increasingly questioning the naturalness of this practice that prevents cow-calf bond and expression of animals' natural behaviors.

In a context of a growing societal concern on livestock farming conditions in general and animal welfare issues in particular, the aim of this doctoral thesis was to experimentally investigate different dairy calf rearing systems allowing cow-calf contacts (CCC). We compared four different CCC practices applied to 14 cows-calf couples, to artificial rearing practices (*Control*) in order to find out the practice corresponding to the best trade-off between animal welfare, animal performance and economic impact.

The first objective was to quantify the consequences of these practices on animal performance and stress at weaning. In Trial 1, we tested two short-time CCC practices, where calves had access to their dams for a short period every day until weaning (20 min "*Before*" and 2.5 h "*After*" morning milking) (Chapter 2). In Trial 2, we tested a day-time CCC, where calves had access to their dam between the two daily milkings ("*Dam*'" 9 h/day). In Trial 3, we tested two day-time CCC; in the first one the calves had a day-time access to dams until weaning ("*Dam"*, 6 h/day) while in the other one the calves had a day-time access to dams until 4 weeks of age before being separated from their dam and reared as calves of the Control group until weaning ("*Mixed"*) (Chapter 3). The second objective was to investigate if the early-life dam-calf contact and grazing experience influence post-weaning behavior and herbage selection of dairy calves in the short term after the turn out to pasture. In Trial 3, we therefore investigated the effect of three different early-life experience on the calves' grazing and social behavior after weaning (Chapter 4). Finally, the third objective was to simulate the economic impact of two suckling practices (*Short-time contact* and *Day-time contact*) on three different farming systems in France (Chapter 5).

Based on the results of this thesis, we can conclude that in our conditions, a short-time CCC immediately after milking fails to cover the calves' nutritional needs and significantly reduces the amount of sellable milk, while a short-time CCC immediately before milking satisfies the calves' nutritional needs but drastically reduces the amount of sellable milk. A day-time CCC for few weeks after birth slightly reduces the milk losses and cows' distress but does not benefit on calves' growth and distress, while allowing a day-time CCC until weaning offers a good compromise between meeting calf nutritional requirements and preserving sellable milk. Dam-calf contact in early-life influences calves' social interactions in both pre-weaning and post-weaning periods and early grazing experience influences the herbage selection of dairy calves in the short term after the turn out to pasture. Distress at weaning is an obvious welfare concern of CCC systems but also of conventional systems for calves, and must be solved by implementing gradual weaning practices. Finally, in terms of economic viability, we concluded that the implementation in dairy systems of a day-time CCC practice during 12 weeks could be an economically viable option as the loss of sellable milk due to suckling is compensated by improved calf growth and reduced workload.

Further studies are still needed to investigate the application of labels that identify "animal welfare" practices or "husbandry systems" traceability to have the opportunity to generate an added value for practices allowing cow-calf contacts.

Riassunto

Nella maggior parte degli allevamenti di bovini da latte i vitelli sono di norma separati dalle loro madri immediatamente o entro poche ore dalla nascita. Sono alloggiati in recinti individuali per 1 o 2 settimane, poi i vitelli maschi sono generalmente venduti per essere ingrassati in aziende specializzate e i vitelli femmine, che saranno le future manze da rimonta, sono allevate in recinti multipli. Lo svezzamento avviene generalmente intorno alle 8-10 settimane di età. La separazione precoce del vitello dalla vacca è un fattore critico dal punto di vista della sostenibilità sociale degli allevamenti bovini, in quanto i consumatori mettono sempre più in dubbio la naturalezza di questa pratica che impedisce il legame vacca-vitello e l'espressione dei comportamenti naturali degli animali.

In un contesto di crescente preoccupazione della società per le condizioni di allevamento in generale e per le questioni di benessere degli animali nello specifico, lo scopo di questa tesi di dottorato è stato quello di studiare sperimentalmente diversi sistemi di allevamento dei vitelli da latte che consentano il contatto vacca-vitello (CCC). Abbiamo confrontato quattro diverse pratiche di CCC applicate a 14 coppie vacca-vitello, con le pratiche di allevamento artificiale (*Control*) al fine di verificare quali tra queste garantisca il miglior compromesso tra il benessere degli animali, le perfomance degli animali e la sostenibilità economica dell'azienda.

Il primo obiettivo è stato quello di quantificare le conseguenze di queste pratiche sulle performance degli animali e sullo stress allo svezzamento. Nella Prova 1, abbiamo testato due sistemi di CCC di breve durata, in cui i vitelli avevano accesso alle loro madri per un breve periodo ogni giorno fino allo svezzamento (20 minuti prima ["*Before*"] e 2,5 ore dopo ["*After*"] la mungitura del mattino) (Capitolo 2). Nella Prova 2, abbiamo testato una pratica CCC giornaliera, in cui i vitelli hanno avuto accesso alla loro madre nel periodo che intercorreva tra le due mungiture giornaliere ("*Dam*", 9 h/giorno). Nella Prova 3, abbiamo testato due pratiche CCC giornaliere; nella prima i vitelli hanno avuto un accesso giornaliero alla madre fino allo svezzamento (*"Dam"*, 6 h/giorno) mentre nell'altra i vitelli hanno avuto un accesso giornaliero alla madre fino a 4 settimane di età prima di essere separati dalla madre e allevati come vitelli del gruppo di controllo fino allo svezzamento ("*Mixed*") (Capitolo 3). Il secondo obiettivo è stato quello di indagare se il contatto precoce tra la madre e il vitello e la precoce esperienza al pascolo influenzino il comportamento post-svezzamento e la selezione dell'erba dei vitelli nel breve periodo dopo il passaggio al pascolo. Nella prova 3, abbiamo quindi studiato l'effetto di tre diverse esperienze nella prima fase di vita sul comportamento al pascolo e sociale dei vitelli dopo lo svezzamento (Capitolo 4). Infine, il terzo obiettivo è stato quello di simulare l'impatto economico di due pratiche di allattamento (contatto di breve durata *"Short-time contact"* e contatto diurno *"Day-time contact"*) su tre diversi sistemi di allevamento in Francia (Capitolo 5).

Sulla base dei risultati di questa tesi, possiamo concludere che nelle nostre condizioni sperimentali, permettere un CCC di breve durata immediatamente dopo la mungitura non riesce a coprire i bisogni nutrizionali dei vitelli e riduce significativamente la quantità di latte vendibile, mentre un CCC di breve durata immediatamente prima della mungitura soddisfa i bisogni nutrizionali dei vitelli ma riduce drasticamente la quantità di latte vendibile. Permettere un CCC diurno per alcune settimane dopo la nascita riduce leggermente le perdite di latte e lo stress allo svezzamento delle vacche, ma non ha benefici sulla crescita e sullo stress allo svezzamento dei vitelli, mentre un CCC diurno fino allo svezzamento offre un buon compromesso tra soddisfare le esigenze nutrizionali dei vitelli e conservare il latte vendibile. Il contatto tra la madre e il vitello nelle prime fasi di vita influenza le interazioni sociali dei vitelli sia nel periodo pre-svezzamento che in quello post-svezzamento e l'esperienza di pascolo precoce influenza la selezione dell'erba dei vitelli nel breve periodo dopo il passaggio al pascolo. Lo stress allo svezzamento è un'evidente preoccupazione per il benessere degli animali nei sistemi CCC

ma anche per i vitelli nei sistemi convenzionali, e deve essere risolta implementando pratiche di svezzamento graduali. Infine, in termini di sostenibilità economica, abbiamo concluso che l'applicazione negli allevamenti di bovini da latte di una pratica CCC diurna durante 12 settimane potrebbe essere un'opzione economicamente sostenibile in quanto la perdita di latte vendibile dovuta all'allattamento è compensata da una migliore crescita dei vitelli e da una riduzione del carico di lavoro.

Ulteriori studi sono ancora necessari per indagare sull'applicazione di certificazioni che identifichino le pratiche di "benessere animale" o la tracciabilità dei "sistemi di allevamento" per avere la possibilità di generare un valore aggiunto alle pratiche che permettono il contatto tra vacche e vitelli.

Résumé

Dans la plupart des fermes laitières européennes, les veaux sont généralement séparés de leur mère immédiatement ou quelques heures après la naissance. Ils sont logés dans des parcs individuels pendant 1 ou 2 semaines, puis les veaux mâles sont généralement vendus pour être engraissés dans des fermes spécialisées. Les veaux femelles, qui seront les futures génisses de remplacement, sont élevés dans des parcs collectifs. Dans les systèmes laitiers, le sevrage correspond à la suppression du lait dans l'alimentation et il est effectué généralement vers l'âge de 8-10 semaines. La séparation précoce du veau de la vache est généralisée dans les systèmes laitiers modernes. Les consommateurs s'interrogent de plus en plus sur le caractère naturel de cette pratique qui empêche le lien vache-veau et l'expression des comportements naturels des animaux.

Dans un contexte de préoccupation sociétale croissante sur les conditions d'élevage en général et sur les questions de bien-être des animaux en particulier, l'objectif de cette thèse de doctorat était d'étudier expérimentalement différents systèmes d'élevage de veaux laitiers permettant de garder le contact la mère et son veau (Cow-Calf Contact, CCC). Nous avons comparé quatre pratiques différentes de CCC appliquées à 14 couples mère-veau, à des pratiques d’allaitement artificiel (*Control*) afin d’identifier la pratique correspondant au meilleur compromis entre le bien-être animal, les performances zootechniques et l'impact économique.

Le premier objectif était de quantifier les conséquences de ces pratiques sur les performances des animaux et le stress au sevrage. Dans l'Essai 1, nous avons testé deux pratiques de CCC de courte durée, où les veaux avaient accès à leur mère pendant une courte période chaque jour jusqu'au sevrage (20 min avant *"Before"* et 2,5 h après *"After"* la traite du matin) (Chapitre 2). Dans l'Essai 2, nous avons testé un CCC, où les veaux avaient accès à leur mère entre les deux traites quotidiennes (*"Dam"* 9 h/jour). Dans l'Essai 3, nous avons testé deux CCC; dans le premier, les veaux avaient un accès quotidien à leur mère jusqu'au sevrage (*"Dam"*, 6 h/jour) tandis que dans l'autre, les veaux avaient un accès quotidien à leur mère jusqu'à l'âge de 4 semaines avant d'être séparés de leur mère et élevés comme les veaux du groupe témoin jusqu'au sevrage (*"Mixed"*) (Chapitre 3). Le deuxième objectif était d'étudier si un contact précoce entre la mère et son veau et l'expérience précoce du pâturage influencent le comportement social et alimentaire des veaux laitiers sevrés lors de la mise à l’herbe. Dans l'Essai 3, nous avons donc étudié l'effet de trois différentes expériences dans la première phase de vie sur le comportement social et alimentaire des veaux sevrés (Chapitre 4). Enfin, le troisième objectif était de simuler l'impact économique de deux pratiques d'allaitement (contact de courte durée *"Short-time contact"* et contact au cours de la journée *"Day-time contact"*) sur trois systèmes d'élevage différents en France (Chapitre 5).

Sur la base des résultats obtenus dans cette thèse, nous pouvons conclure que dans nos conditions expérimentales, une CCC de courte durée immédiatement après la traite ne couvre pas les besoins nutritionnels des veaux et réduit considérablement la quantité de lait commercialisable, tandis qu'une CCC de courte durée juste avant la traite satisfait les besoins nutritionnels des veaux mais réduit considérablement la quantité de lait commercialisable. Un CCC journalier pendant quelques semaines après la naissance réduit légèrement les pertes de lait et le stress au sevrage des vaches mais n'a pas d'effet bénéfique sur la croissance et le stress au sevrage des veaux. En revanche, un CCC journalier jusqu'au sevrage offre un bon compromis entre la couverture des besoins nutritionnels des veaux et la perte de lait commercialisable. Le contact entre la mère et le veau dans la première phase de vie influence les interactions sociales des veaux avant et après le sevrage et l'expérience précoce du pâturage au contact des mères modifie le comportement alimentaire des veaux laitiers sevrés seulement au cours des premiers jours de pâturage. Le sevrage brutal des veaux constitue un problème évident de bien-être pour les veaux et les vaches dans les systèmes CCC mais aussi dans les systèmes

conventionnels pour les veaux. Il devra être résolu par la mise en place de pratiques de sevrage progressif. Nos simulations économiques ont montré que la mise en œuvre dans les systèmes laitiers d'une pratique de CCC journalier pendant 12 semaines pourrait être une option économiquement viable puisque la perte de lait commercialisable due à l'allaitement est compensée par une meilleure croissance du veau et une réduction de la charge de travail.
D'autres études sont encore nécessaires pour examiner la possibilité et l'opportunité de mettre en place des labels identifiant les pratiques respectueuses du "bien-être animal" et permettant de générer une valeur ajoutée pour les pratiques d'élevage des veaux au contact des vaches.

CHAPTER 1

Introduction

General introduction

In most European dairy farms, calves are separated from their dams immediately or within few hours after birth (Busch et al., 2017). They are housed in individual pens for 1 or 2 weeks, then male calves are generally sold to be fattened in specialized farms and female calves, which are the future replacement heifers, are reared in multiple pens. European regulations limits the confinement of the calves in individual cage to 8 weeks of age (Council of the European Union, 2009). Calves are fed milk replacers or non-marketable milk, distributed by an automatic milk feeder or in buckets, and receive increasing amounts of solid feed until weaning (Le Cozler et al., 2012). In dairy systems, weaning corresponds to the suppression of milk in the diet and it generally occurs around 8-10 weeks of age; weaned calves are fed with solid feed, such as concentrates and hay, in increasing amount starting from 2 kg per day.

Maximizing production while reducing costs and labor are the main aims of modern dairy systems. This trend often results in an intensification of farming practices, which weakens societal acceptance of dairy production systems (Cembalo et al., 2016). There is a difference in knowledge between people within and outside the livestock sector. The distance between consumers and farming is growing, most consumers have no direct contact with the farms where their food is produced; consequently, there is a gap between the common morality of consumers and the common morality of farmers (Brom, 2000). At the same time, the number of people who support organizations that defend animal rights and criticize modern farming practices is increasing (Boogaard et al., 2011). In dairy production, the most common welfare concerns are the separation of calves from their dams (Busch et al., 2017) and restricted access to pasture for animals in intensive systems (Schuppli et al., 2014).

Early separation of the calf from the cow is a keystone of modern dairy systems. Citizens question it because it prevents the formation of the cow-calf bond and the expression of animals' natural behavior. On the other hand, some farmers believe that removing calves immediately after birth is the best choice and the less stressful because it prevents the creation of the bond between cow and calf (Flower & Weary, 2001). It allows milking the most milk from the cows and controlling the ingestion of colostrum and milk by calves, it may allow for a better supervision of animals' health by farmers, and consequently it may reduce the risk of transmission of diseases from cow to calf (Flower & Weary, 2001).

At the same time, restricted access to pasture is perceived, especially by people outside the livestock industry, as a deprivation of the expression of animals' natural behavior (Schuppli et al., 2014). Anyway, this topic concerns less people: Schuppli et al. (2014) found that more participants in his study claimed themselves neutral about access to pasture than about the early separation of cows and calves (Ventura et al., 2013). However, it still remains a controversial issue in the dairy industry.

Growing public interest in animal welfare is prompting those who work with farm animals to engage with the public on controversial animal care issues (Ventura et al., 2013). Te Velde et al. (2002) said that farmers feel that the welfare of their animals is good and that they have nothing against animal welfare but they feel uncomfortable with direct or indirect accusations of mistreatment of animals. Kristensen et al. (2008) showed that in farmer' perspectives, animal welfare comes right after team working. The common point, therefore, between the opinion of citizens and farmers is the improvement of animal welfare, which explains why research is also progressing in this direction.

1. Rearing dairy calves: current practices and societal implications

1.1. Maternal behavior and cow-calf bond

Since its domestication that probably occurred around 6000-8000 years ago (Zeuner, 1963), cattle have been used for draft, meat and milk production. More recently, the selection of cows has led to the evolution of a wide variety of breeds distinguished between beef and dairy breeds. Certain aspects of maternal behavior are considered relevant to both of these production systems, as they play an important role on offspring survival during the pre-weaning period (Grandinson, 2005). In dairy cattle for example, since the cow has to release milk in the absence of her calf and has to be accustomed to early handling and separation from the calf, only certain aspects of maternal behavior have been selected (Edwards & Broom, 1982). In this case, among the characteristics of maternal behavior, the selection of the ability to create a strong bond with the offspring is less prioritized because in intensive production systems the calf is separated from the dam a few hours after birth. However, even in intensive systems there is a growing interest in exploiting the capacity of the dam to care successfully for her offspring without human intervention, in order to reduce labor (Grandinson, 2005). The early separation of the calf from its dam stimulates the return to the reproductive cycle by dams and allows farmers to improve the performance of the offspring in their breeding population (Newberry & Swanson, 2008).

In beef cattle, the ability to nourish and take care of the offspring and protect them from predator attacks, is selected to ensure a high survival and growth rate in the offspring (von Keyserlingk & Weary, 2007). In extensive beef cattle systems, the calves are keep with their dams and weaned at around 6 months of age (Enríquez et al., 2011).
More extensive dairy systems, including organic farming, could adopt different forms of cow-calf contact to take advantage of the benefits for calves of having access to maternal care, learning, and socialization (Mogi et al., 2011).

The maternal behavior plays a fundamental role in the survival and the growth of the offspring in many species. In pig production, for example, good maternal behavior is an important criterion as the increase in litter size at birth due to genetic selection requires mothers to be able to rear large litters (Grandinson, 2005). Mortality in piglets from birth to weaning generally amounts to 16-20%, with the main causes attributed to stillbirth, crushing and starvation (Edwards & Baxter, 2015). Moreover, aggression, rejection or lack of interest in offspring could compromise the survival of the newborns and it is therefore important that the bond between mother and offspring is created at birth. Because of the size of the litter, the mother-young bond between sow and piglets is less strong than in species with lower progeny, the survival of the piglets depends mainly on birth weight and not only on the selectivity of the sow (Souza et al., 2014), although it has been shown that sow can be selective towards foster piglets older than 1 to 2 d of age (Price et al., 1994).
Ewes, like cows, generally have 1 or 2 offspring, which makes them more selective than sows. As reported by Holmes et al. (1989), ewes are less likely to accept a foster lamb if they do not have their own lamb's odor (an odor-transfer 'add-on' fostering technique, where foster lambs wear a jacket impregnated with the natural odor of their own lamb) and have a tendency to accept as many lambs as they have lambed.

The social bond between a mother and her newborn can be 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 behavior such as allogrooming, provision of nourishment, warmth and protection, resting in contact, synchronizing activities, and maintaining proximity. In other species, such as pigs for example, sow does not lick her piglets at birth, but often piglets seeks nose-to-nose contact with the sow just before the first suckling (Grandinson 2005).

Often dams with their calves synchronize their activities more than unrelated animals in the same group (Veissier et al., 1990). Bonded individuals exhibit reinstatement behavior when motivated to reunite after a period of separation, and greeting behavior upon reunion (Newberry & Swanson, 2008). Maintaining close proximity between mother and young allows for social transmission of information such as food sources and predators (Thorhallsdottir et al., 1990).

Under natural conditions, the first hours after birth are essential for the creation of a bond between dam and calf. The time required for bond formation is called as the 'critical period' (Estes, 1971). If this relationship is not created, the calf could be rejected by his dam. In addition, the latency to first suckling determines the level of passive immunity achieved by the neonate and thus its susceptibility to contract infectious diseases (Edwards & Broom, 1982). Under natural condition, the safety of the newborn totally depends on this. Lidfors et al. (1996) found that calves immediately separated from their dam after birth had lower amount of antibodies in their blood than calves licked from their dam. Therefore, at birth, it is necessary for the cow to lick the calf. The behavior of licking has numerous vital functions: it allows removing the foetal membranes, to dry the calf and reduce the dispersion of body heat and to stimulate the newborn activities such as breathing, circulation, defecation and urination. This stimulation boots the increasing neuro-excitability and consequently promotes rapid motor development in the young, increasing its chances for survival (Estes, 1971). The development and maintenance of maternal attachment is provided through the presence of hormones such as oestradiol, oxytocin and prolactin, released shortly after birth, which also promote the identification of the calf by stimulating its individual recognition and memorization, in particular by the smell (Enríquez et al., 2011). Initial maternal attachment could occur within a few hours after parturition, while the establishment of a strong mutual bond could take several days (Newberry & Swanson, 2008).

For species that live in social groups that include multiple couples of mothers and young, it is important that mother and young have a bond based on mutual recognition to ensure that the mother selectively cares for her own young (Gubernick, 1981). The mutual recognition involves all the senses, in particular hearing: after only the first 24 hours after calving, the calf is able to recognize the mother simply by the sounds she emits; indeed the frequency and duration of the vocalizations are specific to each pair (Enríquez et al., 2011).

In nature, therefore, the bond between mother and young is crucial and could last for a long time, while in the management of captive animals the two are separated prematurely, in a brutal and permanent way and often before the natural phase of weaning (Newberry & Swanson, 2008).

1.2. Rearing dairy calves: current practices

In 2020, 34,595 million cattle were reared in Europe of which 22,395 million were dairy cows (L'économie laitière en chiffres, 2021). In 2020, in France, out of 18,151 million cattle, 3,970 million are beef cows, 3,486 million are dairy cows, and the rest are veal calves (L'économie laitière en chiffres, 2021). Also in France, in 2019, the number of dairy cows reared under organic specification or in conversion reached about 250 000 individuals, i.e. 7% of the total (10% more than in 2018) (Agence Bio, 2020).

In French and most of European dairy farms, calves are separated from their dams immediately or within few hours after birth (Busch et al., 2017). They are housed in individual pens for 1 or 2 weeks, then male calves are generally sold to be fattened in specialized farms and female calves, which are the future replacement heifers, are reared in multiple pens. European regulations limit the confinement of the calves in individual cage to 8 weeks of age. The Council Directive 2008/119/EC (December 2008, as amended, since 1st January 2007) *"prohibits the use of confined individual pens after the age of eight weeks. The Directive, amongst other things, sets out minimum dimensions for individual pens and for calves kept in group. Calves are not to be tethered (except under very specific circumstances) and must be fed according to their physiological needs. In particular their food must contain sufficient iron and a minimum daily ration and fibre food must be provided."*

Calves are generally fed with milk replacers or non-marketable milk, distributed by an automatic milk feeder or in buckets, and receive increasing amounts of solid feed until weaning (Le Cozler et al., 2012). The main reasons and arguments why the practice of early cow-calf separation immediately after birth is so widely accepted as a normal practice of professional dairy farming are:

- *<u>Health/Sanitary reasons</u>*: Separating the calf from the dam within 1 to 2 hours of birth allows controlling colostrum ingestion during the critical absorption period (Godden, 2008) and may reduce the risk of pathogen exposure or disease transfer from the dam (Windsor & Whittington, 2010).
- *<u>Economic reasons</u>*: Preventing the calf from suckling provides more milk for the farmer and the loss of sellable milk is lower if the calf is not fed *ad libitum* (Meagher et al., 2019). Also most farmers feed calves with 'waste' milk or milk replacers which are less expensive than fresh milk (Flower & Weary, 2003) and there is a, increased risk of reduced milk let-down reflex efficiency (Kilgour & Dalton, 1984) which could result in increased milking time (Flower & Weary, 2003) when the calf suckles.
- *<u>Distress reasons:</u>* Early separation is considered to be less stressful than late separation because it prevents the creation of the bond between cow and calf (Flower & Weary, 2001). Early separation leads to easier handling of the calf, accustoming it to human contact and reduces the risk of aggression of the dam towards the farmer (Vaarst et al., 2020).

Weaning corresponds to the suppression of milk in the diet and it generally occurs around 8-10 weeks of age, often in an abrupt way; weaned calves are fed with solid feed, such as concentrates and hay, in increasing amount starting from 2 kg per day.

In commercial dairy farms, calves rarely experience grazing during their early lives (Costa et al., 2016). In Le Cozler's study (2012), 60 % of dairy farms use seasonal batch calving during autumn and winter, in order to turn out animals to pasture in the following spring. Then, calves and heifers usually graze from spring to autumn, before their first year of age, but only 2% of dairy farms turn out calves to pasture before 6 months of age (Le Cozler et al., 2012).

Regarding reproduction, over 98% of farmers use artificial insemination (AI) (Le Cozler et al., 2012). Most farmers have a target age for first calving at 24 months (44%), 30 months (42%) or 36 months (10%). Farmers who begin breeding heifers earlier than 15 months, use more concentrates during the first year of the heifers than those who choose late calving. Heifers with later calving (36 months) generally do not receive feed supplements: in addition to grazing, feeding is based on corn silage, hay, and mineral supplements in winter (Le Cozler et al., 2012).

Furthermore, in Le Cozler et al.'s (2012), 62% of the farmers surveyed believe that rearing heifers is a necessity, 36% find it not only necessary but above all enjoyable, while 2% believe it is simply part of their routine.

1.3. Attitude towards early cow-calf separation and zero-grazing dairy systems

In the last 50 years, animal production systems in Europe have changed considerably in order to respond to increased demand while reducing costs, so it has been necessary to increase the efficiency of production through the modernization and the automation of farming systems (Lassen et al., 2006). This led to an intensification of production by selecting animals that are more productive, increasing the number of animals and reducing the space per individual. In addition, the increase in efficiency caused the disappearance of many small extensive farms giving way to large intensive farms (Fraser, 2008). Thus, a secondary effect of these changes has been a drastic reduction in the number of people involved in animal production (Lassen et al., 2006) and a distancing and detachment of the public from this reality. Only starting from the late 20th century, public awareness grew about animal welfare and what is done to animals in intensive animal production (Dumont et al., 2016; Fraser et al., 1997). People are increasingly interested in how their food is produced (Cembalo et al., 2016). Several studies investigated public opinion regarding common practices on farms and animal welfare (Cardoso et al., 2016; Hötzel et al., 2017; Te Velde et al., 2002; Ventura et al., 2016). First, the public is often unaware of common farming practices (Ventura et al., 2016) but what has been found is that people interviewed in the studies, after being made aware of the practices used on farms, still continue to reject some of them (Hötzel et al., 2017; Ventura et al., 2016). This suggests that the education to livestock farming may resolve some concerns but others will persist because it conflicts with core values regarding animal care (Ventura et al., 2016). In particular, Hötzel et al. (2017) found that practices that are difficult for the public to accept in dairy production are the early separation of the calf from its dam and the limited access to pasture. Animal welfare and loss of naturalness were the main reasons why participants rejected both zero-grazing and early separation, those who rejected zero-grazing were concerned about the impact of production practices on product quality and thus human health. In general, the shared common opinion reported was that cows should be fed like ruminants and young mammals should be cared by their mothers, other realities would be aberrant (Hötzel et al., 2017).

Cardoso et al. (2016) also reported that for public opinion, animal welfare is summed up in the quality of life of the animals, which consequently leads to a better quality of milk or animal products. However, in contrast to the public, farmers tend to express less concern over animal welfare (Vanhonacker et al., 2008). As found by Te Velde (2002), farmers' perception of animal welfare is generally positive, according to them there are no problems of welfare in livestock systems. The concept of animal welfare for many farmers is based on physical health: if an animal is healthy, eats and grows fast it means that it is *well*, while for the public it is important that the animal *feels* good, i.e. it has a certain psychological well-being (Te Velde et al., 2002). In addition, farmers have a more professional and technical point of view. Schuppli et al. (2014) have shown that some farmers and veterinarians do not consider grazing only as a natural life experience, but on the contrary, they believe that grazing can be a source of stress for the animals. Confined animals can have every comfort, such as food, water, weather protection, while grazing animals, especially highly productive ones, can face several difficulties such as the inability to respond to their high nutritional requirements.

Concerning the cow-calf early separation, Placzek et al. (2020) have shown that the opinion of most farmers is mainly economic, since in dairy production, milk is the main source of income. Keeping calves with their dam would reduce the amount of saleable milk and consequently the income of farmers. In addition, farmers point out that the early separation between cow and calf is less stressful than a later separation because the time necessary to create a bond it is not given (Flower & Weary, 2001; Ventura et al., 2013).
These different perceptions on animal welfare show that there is no unified "public opinion" on farming practices (Busch et al., 2017).

Generally, the people involved in dairy sector accept more easily the practices criticized by those who are outside of it (Ventura et al., 2013). However, common concerns about early separation were found between the two parties, e.g. both farmers and people not involved in the dairy sector showed concern about calf welfare as emotional distress and health and farmers showed a willingness to change their practices if a reasonable compromise can be found (Ventura et al., 2013).

1.4. Animal welfare

The growing public concern for animal welfare has generated the demand for the development of an animal welfare science that acts to improve animal welfare (Webster, 2016). The definition of animal welfare can be summarized as follows: *the welfare of any sentient animal can be defined as its perception of itself on its physical and mental state, i.e. if it stays fit and happy* (Webster, 2001).
Therefore, the new approach used by animal welfare science has been to ask animals what matters to them and how much it does matters (Dawkins, 1993).

Thanks to the combination of the scientific approach and the identification of animal needs, it has been possible to develop the first principles of animal welfare that take into account all phases and aspects of the life of livestock animals, from the farm, through the transit to the time of slaughter (Webster, 2001).
Franklin Roosevelt first introduced the concept of four freedoms in his speech to the US Congress in 1941, referring *to freedom of speech, freedom of worship, freedom from want and freedom from fear* (Webster, 2016). This principle was later echoed in the Brambell report (*Report of the Technical Committee to enquire into the welfare of animals kept under intensive livestock husbandry systems,* 1965) concluding that livestock animals should have sufficient space to perform five behaviors or activities, such as getting up, lying down, turning around, stretching their limbs, and grooming their self (Webster, 1995).
In 1993, with the collaboration of John Webster, the Farm Animal Council (FAWC) published an updated version in which each of the five freedoms was combined with five provisions (Farm Animal Council, 1992; Webster, 2016):

The Five Freedoms and Provisions
1. *Freedom from thirst, hunger and malnutrition*
   – by ready access to fresh water and a diet to maintain full health and vigor.
2. *Freedom from discomfort*
   – by providing a suitable environment including shelter and a comfortable resting area.
3. *Freedom from pain, injury and disease*
   – by prevention or rapid diagnosis and treatment.
4. *Freedom to express normal behavior*
   – by providing sufficient space, proper facilities and company of the animal's own kind.
5. *Freedom from fear and distress*
   – by ensuring conditions which avoid mental suffering.

The five freedoms take into account both physical and mental well-being. The primary idea behind the five freedoms is not to eliminate stress but to prevent animals from suffering, or at least suffering unnecessarily (Webster, 2001). Therefore, it does not mean that animals should never be exposed to stress, but that they have the tools (physical and mental well-being) to solve potentially stressful problems, such as hunger, thirst, heat/cold, fear, frustration, etc. (Webster, 1995).
The five freedoms have formed the basis for regulations or specifications aimed at protecting animals (for example, the Freedom Food Scheme in the United Kingdom). The Welfare Quality® project has clarified these five freedoms by breaking them down into 12 welfare criteria, then proposing indicators for each criterion, indicators that can be measured on the farm or at the slaughterhouse (Dumont et al., 2016) (Table 1.1).

Table 1.1. Principles, criteria, and measures of the Welfare Quality® assessment protocol for cattle (2009) (adapted from Wagner et al. 2021).

Principles	Criteria	Measures
Good Feeding	1. *Absence of prolonged hunger*	Body condition score
	2. *Absence of prolonged thirst*	Number, functionality and cleanliness of water points
Good Housing	3. *Comfort around resting*	Difficulties rising or lying, cleanliness of the animal
	4. *Thermal comfort*	As yet, no measure is developed.
	5. *Ease of movement*	Presence of tethering, access to outdoor loafing area or pasture
Good health	6. *Absence of injuries*	Lameness, integument alterations
	7. *Absence of disease*	Coughing, nasal discharge, ocular discharge, hampered respiration, diarrhea, vulvar discharge, milk somatic cell count, mortality, dystocia, downer cows
	8. *Absence of pain induced by management procedures*	Disbudding/dehorning, tail docking
Appropriate Behavior	9. *Expression of social behaviors*	Agonistic behaviors (head butts, displacements)
	10. *Expression of other behaviors*	Access to pasture
	11. *Good human-animal relationship*	Avoidance distance,
	12. *Absence of general fear*	Qualitative behavior assessment (defined by 20 terms of body language)

Thanks to this classification method, it is possible to identify the most problematic points in the different types of farming systems. These problems are generally not specific to any kind of system and there are significant variations between farms within systems, depending on housing conditions, practices and farmer behavior, so these criteria allow defining and evaluating animal welfare in any livestock context.

The lack of welfare and many individual animal welfare abuses may also be attributed to poverty, ignorance, or neglect (Webster, 2001): Ensuring proper remuneration to farmers gives them the opportunity to implement basic rules to ensure animal welfare.

The creation of labels that protect and valorize animal welfare is still not very widespread, but organic production goes in this direction. This is where the controversy between the public's desire to improve animal welfare standards and the desire to pay for products that provide welfare at a higher price comes in (Webster, 2001).

1.5. Organic agriculture, farming and dairy production

Beginning in the 1920s, in Austria, Germany, Switzerland and in England, and later since the 1970s in France as well, a sub-cultural movement opposed agricultural industrialization (Lund, 2002), questioning its dominant model, criticizing the use of agro-chemistry that destroyed soils and showing

human health concerns due to the consumption of products from this production system (Touret, 2015).
The early organic farming movements were also critical of industrialized animal husbandry, they questioned about animals welfare and aspired to develop more sustainable, environmentally and animal friendly farming systems (Lund, 2006).
The organic agriculture appears officially in 1991 in a European regulation (EEC n° 2092/91), that concerned only vegetable and processed products. A regulation on livestock production was published later in 1999. Organic agriculture has seen growth and expansion in all member states. This led the European Commission to draw up a regulation to protect and to control the production and the labelling of organic products (EC n° 834/2007), in force since 2009 and concerning all member states:

"Organic production is an overall system of farm management and food production that combines best environmental practices, a high level of biodiversity, the preservation of natural resources, the application of high animal welfare standards and a production method in line with the preference of certain consumers for products produced using natural substances and processes (EC n° 834/2007)*."*

The institution of the European organic label is one of the main innovations introduced by this legislation, adopted in 2007 by the agriculture ministers of the member states after a year and a half of negotiations. The European organic label was created by the European Union to make it easier for European consumers to recognize "organic" products (Schmitt 2018).

The word "organic" is used nowadays as a synonym for "natural, healthy" but it is not always used appropriately. The definition of "organic animal husbandry", on the other hand, is very strict and defines livestock productions that adhere to the IFOAM Basic standards for Organic Agriculture and Food Processing (Lund, 2002). IFOAM is the acronym for the International Federation of Organic Agricultural Movements and is responsible for the regulation of organic standards, which are the most widely used throughout the world:

"*Organic livestock husbandry is based on the harmonious relationship between land, plants and livestock, respect for the physiological and behavioural needs of livestock and the feeding of good-quality organically grown feedstuffs* (IFOAM, 2006)."

The main rules concerning organic dairy production are detailed in Table 1.2. The transition time to switch a dairy operation from conventional dairy production to organic dairy production, is two years. The obtainment of 'organic' certification is submitted to the adhesion to a certifying organization that carries out annual controls. The EU law-makers require all farms to be inspected when applying for organic certification (EC art. 34 2018/848) and they are also "subject to a verification of compliance at least once a year" including "a physical on-the-spot inspection" (Duval et al., 2020).

Table 1.2. List of the main practical measures for the application of the organic regulations for dairy cows (Regulations (EC) n°834/2007, (EC) n° 889/2008) and (EC) 2018/848).

	Obligations	**Suggestions**
Origins of animals	Organic livestock shall be born and raised on organic holdings. Non-organic adult male and nulliparous female mammals, may be introduced into the herd (up to a maximum of 10 % of the herd, respecting the transition period of 6 months)	Prefer breeds adapted to local conditions, with high genetic diversity, ensuring a high level of animal welfare and helping to prevent any suffering and to avoiding the need for the mutilation of animals

Housing condition	It is required to satisfy the specific needs of the animal, including its behavioral needs. Individual pens for calves over one week old are prohibited	The livestock building can be improved and enriched with mineral products (listed in Annex I (EC) No 834/2007)
Access to pasture	Access to organic pastures whenever weather conditions permit	Access to the pasture is not mandatory during the winter season.
Calf feeding	Calves must be fed with milk produced on the farm for at least 3 months	Preferably maternal milk
Disease prevention	The preventive use of chemically-synthesised allopathic medicinal products is not permitted	Vaccines can be used
Veterinary treatment	Under the responsibility of the veterinarians, a maximum of 3 chemically synthesised allopathic medicinal products can be used per animal. It is required to double the withdrawal period of minimum 48 hours	Phytosanitary and homeopathic medical products are to be preferred if they have a real therapeutic effect on the animal concerned and on the condition for which the treatment is required

Cow-calf contact systems are not considered in the regulation. The guidelines for the interpretation of the measures specified, since 2013, that calves older than one week must be housed in pens that can accommodate several animals in conformity with the surface areas of Annex III (EC) No 889/2008. The French Comité National de l'Agriculture Biologique (CNAB), which is in charge of interpreting the European organic regulations, with regard to the issue of outdoor access and grazing for organic calves, stated that starting from January 1, 2022:

« Calves must have access to outdoor spaces as soon as possible and no later than 6 weeks of age except during the winter period […] in accordance with 1.9.1.1(d). » (Parrain, 2020).

This new interpretation could be a reason for an increase in rearing systems that allow for cow-calf contact (Vaarst et al., 2020).

2. Cow-calf contact in dairy systems

2.1. Definitions

The conventional rearing system, in which calves are separated from their dam a few hours after birth and then are artificially fed with milk and milk replacers, is called **artificial rearing systems**.
Instead, systems that allow physical contact between a dam and her calf or a foster cow and her foster calf are called cow-calf contact systems (CCC) (Sirovnik et al., 2020) (Figure 1.1).
More specifically, it is defined as **dam-calf contact rearing** a system that allows physical contact between the dam and her own calf, while it is called **foster cow rearing** when a cow nursed one or more foster calves (Sirovnik et al., 2020). Usually the foster cow is not milked but it depends on her stage of lactation and on the number of nursed calves (Johnsen et al., 2016).
A study conducted by Sirovnik et al. (2020) on the terminology and definitions of different cow-calf contact systems categorized them according to the *type of physical contact* and *duration of contact*:

- *Type of physical contact*

The different types of physical contact include **full cow-calf contact**, where physical contact between cow and calf is allowed without restriction, and **partial cow-calf contact**, where the contact can be restricted by fence-line, or by the application of nose-flap to calves or udder nets to cows.

- *Duration of contact*

Whole day cow-calf contact indicates that the cow and the calf are allowed to have physical contact for at least 24 hours, with the exception of the time when the cow goes to the milking parlor or to the feeding alley, if provided.

Partial-time cow-calf contact, when the duration of the separation is longer than the milking or the feeding period and is imposed by human. In turn, it can be defined as:

short-time cow-calf contact when cow-calf contact is allowed once or more time per day, usually around milkings, for short periods, e.g. 15 minutes after 2 milkings (Margerison et al., 2002) or 30 minutes after 2 milkings (Mendoza et al., 2010);

day-time or night-time cow-calf contact, when contact is expected for longer periods but less than 24 hours, during the day-time or night-time.

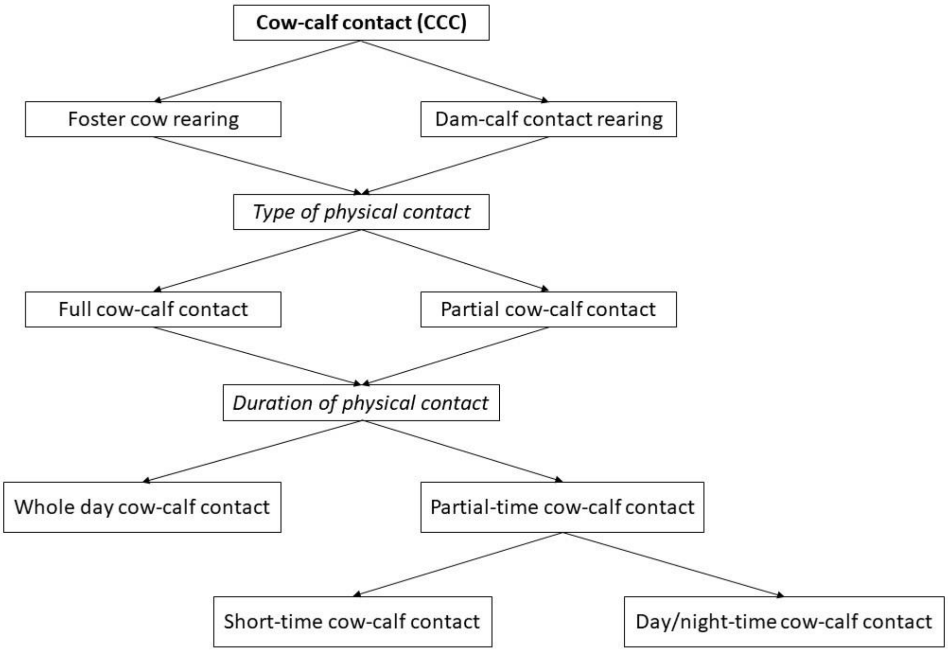

Figure 1.1. Summary flowchart on definitions of cow-calf contact systems.

The different cow-calf contact rearing systems have developed from the practical experience of pioneer farmers who keep cows and calves together (Johnsen et al., 2016). Several European countries apply this practice (Vaarst et al., 2020). In Norway and Sweden, 18% and 22% of organic dairy cow farmers, respectively, keep calves with their dams during the first week of life up to 13 weeks for some farmers (Ellingsen et al., 2015). In France, Le Cozler et al. (2012) reported that only 4% of farmers keep calves with their dam at later than 24 h, but that this practice is increasingly used. Also Michaud et al. (2018) investigated farms using a suckling practice in France (Massif Central, East and West of France), and found that 62 farms out of 102 kept calves with their dam or with a foster cow between 1 and 60 days of age.

In the following sections, the consequences (advantages and disadvantages) of cow-calf contact systems in terms of calves and cows' performance, behavior and health will be discussed in detail.

2.2. Consequences of cow-calf contact on calves' performance

2.2.1. Calves' growth

One of the main expected benefits of allowing calves to suckle freely their dam is a high growth rate of these latter (Fröberg et al., 2011). In Krohn et al. (2001)'s review it is reported that in most studies that allowed calves to suckle, the calves were healthy and with a high weight gain.
However, a better growth of suckling calves compared to artificially reared calves may also depend on the type and duration of cow-calf contact.
Several studies, that investigated full cow-calf contact during whole day, found an increased weight gain of suckling calves compared to artificially reared calves. For instance, Metz (1987) found a higher weight gain at two months of age when the suckling period lasted only 10 days, and a whole day cow-calf contact was provided, compared to calves bucket-fed with 8 kg of milk replacer day $^{-1}$. Fröberg et al. (2011) found that free suckling during 8 weeks led suckling calves to have a higher weight gain than artificially reared calves fed with high quantity of milk (9 L/d with automatic teat-feeding). Roth et al. (2009) compared two suckling systems to two artificial systems (8 L/d with automatic milk feeder, 2 times daily one and 6 times daily the other) and found that calves with unrestricted suckling had the same growth rate as calves with short time contact (2 x 15 min before the two milkings), both gained significantly more weight during the milk feeding period than artificially reared calves.

However, some studies also found that calves suckled by their dam grew more slowly than artificially reared calves, even if suckled calves had whole day cow contact (Veissier et al., 2013).
Systems that allow a partial time of cow-calf contact showed results that were often contradictory. Margerison et al. (2002) found that calves suckled twice daily for 15 min after milkings did not allow suckling calves to benefit in terms of growth rate, at least during the milk-feeding period. Fröberg et al. (2005), let calves suckle 3 times a day for 2h after the two milkings and found that calves buckets fed in single pen gained more weight. While Mendoza et al. (2010) found that restricted suckling calves for twice a day for 2h after milking had a higher weight gain than calves buckets fed twice daily.
These different results depend on many factors, but in general, the longer the duration of cow-calf contact is, the better the performance of the calves are in terms of growth rate. This may be because unrestricted calves ingest more milk than restricted calves. Studies in which the amount of milk ingested was measured, showed that good growth rates were correlated with high milk ingestion and vice versa (Bar-Peled et al., 1997; Fröberg et al., 2005; Margerison et al., 2002; Mendoza et al., 2010; Teeluck et al., 1981; Table 1.3).
However, it is not only the amount of milk ingested that influences growth, the presence of the dam also has beneficial effects. Krohn et al. (1999) compared growth rates and behavior of calves separated immediately after birth and 5 days after, and concluded that social interaction between cow and calf during the colostrum period had a positive effect on the daily gain of the calf. Also, Lupoli et al. (2001) found an increase of oxytocin in suckling calves compared to calves bucket fed and showed that this hormone influences anabolic processes and growth. They also correlated the increase of oxytocin with the decrease of the level of cortisol, which means that suckling have an anti-stress effect in both cows and calves, so they concluded that it is not only suckling that determines this effect but the presence of the cow or calf respectively.

During the milk feeding period, suckling calves often ingest small quantities of solid feed, in fact it is known that a high milk intake is associated with low grain intake (Johnsen et al., 2015; Roth et al., 2009). A high daily gain achieved through a high milk intake is not always beneficial, because it results in a decrease of roughage intake, and thus a delay in rumen development which increases the difficulties associated with weaning or separation (Krohn, 2001). Systems that provide a separation period between cow and calf during the milk feeding period, could encourage calves to ingest solid feed when they are separated from their dam, and this may have positive effects especially during the first weeks after weaning (Fröberg et al., 2011).

Finally, one of the main negative effects of cow-calf contact on calves' growth is the weight loss after weaning (this topic will be discussed more in detail in a following section). Most studies on calves growth after weaning have found that weaning has a stronger impact on daily weight gain of dairy calves than of artificially reared calves (Bar-Peled et al., 1997; de Passillé et al., 2008; Hepola et al., 2007; Table 1.3). This disadvantage can be reduced if weaned calves are more aged (12/13 weeks old), in this way the time needed for calves to be accustomed to solid feed as diet is reduced and thus also the negative effects on weight gain (de Passillé et al., 2011).

2.2.2. Calves' behavior

Suckling is the most important behavior in the reproductive process of mammals because it allows the transfer of milk from mother to the young.
However, in conventional rearing systems, calves are fed milk with bucket or automatic milk feeder and this does not allow them to perform natural suckling behavior, even if an artificial teat is provided (Roth et al., 2009). Thus, to satisfy the motivation to suckle, calves may develop different behavioral disorders such as excessive licking on objects or suckling on body part of other calves (de Passillé, 2001; Veissier et al., 2013), and this can lead to the transmission of diseases (de Passillé, 2001) or the increase of risk of disorders such as navel abscesses, drinking urine etc. (Veissier et al., 2013).
Non-nutritive oral activities, such as cross-suckling, linking and tongue-rolling may be related to insufficiently stimulated or suppressed feeding behaviors (Veissier et al., 2013).
Roth et al. (2009) showed that calves allowed suckling their dam only for 15 min twice daily did not show cross-suckling compared to artificially reared calves (fed with a similar number of whole milk meals) that performed it. Also Fröberg et al. (2009) confirm that free suckling calves did not performed tongue-rolling during the milk feeding period compared to artificially reared calves. This shows that artificial milk feeders do not satisfy completely the motivation to suckle of calves (Roth et al., 2009).
In the study of Johnsen et al. (2015), calves were allowed to suckle their dam at night and were feed ad libitum from an automatic feeder throughout the day and night. They found that calves, even though they had the option of drinking milk from an automatic feeder throughout the day, preferred nursing to drinking from an automatic feeder.
Only the presence of the dam can influence positively the development of calf specific behaviors and can help it to focus its oral activities toward nutritive elements, such as milk or solid feed and toward natural behaviors such as suckling and licking its dam (Veissier et al., 2013) (Figure 1.2).

The presence of the dam in the early stages of a calf's life can also have positive effects on its social interactions. Le Neindre & Sourd (1984) found that dam-reared calves had higher levels of social activity than calves separated immediately after birth. Krohn et al. (1999) showed that even 5 days of cow-calf contact without suckling had a positive impact on social learning of calves, while suckling seemed important to the establishment of the social bond, both the presence of the dam and suckling decreased the fearfulness of other animals. Keeping calves with their dam during the first days after birth may allow calves to develop a more comprehensive social repertoire that could help them if they are eventually introduced into a group (Flower & Weary, 2001).
This suggested that even the first days of life are crucial not only for social bonding with the dam but also for calf's social learning. Nevertheless, it may also increase the calves' fear of humans (Krohn et al., 1999).

2.2.3. Calves' health

In addition to abnormal oral behaviors, diseases are a main problem in artificial rearing systems.
During the milk feeding period calves are more exposed to respiratory diseases and diarrhoea. The presence of these infectious diseases during the first 90 days of life may negatively affect the performance of the animal in adulthood (Svensson et al., 2003).
A common belief is that separating the calf at birth limits the risk of transmission of diseases and is better for both calf and cow health. Artificial feeding of calves is thought to better control the quantity and the quality of colostrum ingested allowing good transfer of maternal immunoglobulins to the calf (Beaver et al., 2019). Transmission of enteric pathologies from dam to calf occurs mainly via fecal-oral transmission through the colostrum or the environment (McGuirk, 2008). Moreover, during the periparturient period the dam's fecal coliform bacterial count increases (Pelan-Mattocks et al., 2000; cited by Beaver et al., 2019) and this justifies the concern that keeping calves in the calving area increases the risk of exposure to pathogens (McGuirk, 2008).
However, studies on the consequences of suckling on the health of calves during the early stages of life reported contrasting results.
Weary & Chua (2000), Metz (1987) and Boonbrahm et al. (2004) showed a beneficial effect of the dam' presence on calves' health. Weary & Chua (2000) reported that calves that spent more time with dam (4 days after birth compared to 1 day and 6 h after birth) had fewer bouts of diarrhoea during the first 3 weeks of life; Boonbrahm et al. (2004) found that bucket-fed calves had a higher mortality (15%) compared with dam-reared calves (0%). Franklin et al. (2003) and Krohn et al. (1999) reported no difference on the incidence of diseases between free suckling calves and artificially reared calves. Roth et al. (2009), Margerison et al. (2002)and Fröberg et al. (2005), on the other hand, indicated that dam-calf contact increases the risk of infection. Roth et al. (2009) hypothesized that the higher quantity of milk consumed by suckling calves could have increased the incidence of diarrhoea, but they concluded that the health state of calves could not be improved by the presence of the dam.
Finally, Beaver et al. (2019) reviewed the available literature and found no evidence that suckling has negative effects on health. For instance, when latency to first suckling and quality of colostrum are controlled, leaving the calf with its dam at birth has no effect on risk for diarrhoea or on mortality (Beaver et al., 2019).

Figure 1.2. Two-week-old calf learning to eat hay from a cow, at INRAE 'Herbipole' experimental farm (Marcenat, Fr).

In conclusion, we can say that cow-calf contact systems could be more beneficial to the welfare of calves compared to artificial rearing systems (Krohn 2001). Keeping calves with their dam allows performing natural behavior such as suckling and social bonding with dam, learning to eat roughage earlier, having social contact with other calves and cows, and have potential positive effects on growth without apparent negative health impacts.

Table 1.3. Advantages (+), disadvantages (-) and similarities (=) of different CCC on calves' performance and feed intake (milk and concentrate) before and after weaning, compared to artificial rearing systems.

			Whole day cow-calf contact	Duration		Short-time cow-calf contact	Duration		Day-time or night-time cow-calf contact	Duration
Calves' performance	*Milk intake during suckling period*	+	Metz et al. (1987)	**1.5 wk**	+	Bar-Peled (1997)	**6 wk**			
					-	Froberg et al. (2005)	**8 wk**			
					-	Margerison et al. (2002)	**26 wk**			
					+	Mendoza et al. (2010)	**8 wk**			
					-	Teeluck et al. (1981)	**12 wk**			
	Concentrate intake during suckling period	+	Flower & Weary (2001)	**2 wk**	-	Bar-Peled (1997)	**6 wk**			
		-	Froberg et al. (2011)	**8 wk**	-	Margerison et al. (2002)	**26 wk**			
		-	Roth et al. (2009)	**13 wk**	=	Froberg et al. (2005)	**8 wk**			
					-	Roth et al. (2009)	**13 wk**			
	Growth during suckling period	+	Flower & Weary (2001)	**2 wk**	+	Bar-Peled (1997)	**6 wk**	+	Johnsen et al. (2015a)	**6 wk**
		+	Froberg et al. (2011)	**8 wk**	+	de Passillé et al. (2008)	**8 wk**	=	Veissier et al. (2013)	**10 wk**
		+	Metz et al. (1987)	**1.5 wk**	-	Froberg et al. (2005)	**8 wk**			
		+	Roth et al. (2009)	**13 wk**	=	Froberg et al. (2007)	**16 wk**			
		=	Veissier et al. (2013)	**10 wk**	-	Margerison et al. (2002)	**26 wk**			
					+	Mendoza et al. (2010)	**8 wk**			
					+	Roth et al. (2009)	**13 wk**			
					-	Teeluck et al. (1981)	**12 wk**			
	Growth at weaning	+	Froberg et al. (2011)	**8 wk**	+	Mendoza et al. (2010)	**8 wk**			
	Concentrate intake after weaning	-	Roth et al. (2009)	**13 wk**	+	Hepola et al. (2007)	**5 wk**	+	Johnsen et al. (2015a)	**6 wk**
					-	Froberg et al. (2005)	**8 wk**			
					-	Froberg et al. (2007)	**16 wk**			
	Growth after weaning	-	Froberg et al. (2011)	**8 wk**	-	Bar-Peled (1997)	**6 wk**	+	Johnsen et al. (2015a)	**6 wk**
		-	Veissier et al. (2013)	**10 wk**	-	de Passillé et al. (2008)	**8 wk**	-	Veissier et al. (2013)	**10 wk**
		-	Roth et al. (2009)	**13 wk**	-	Hepola et al. (2007)	**5 wk**			
					+	Margerison et al. (2002)	**26 wk**			
					-	Roth et al. (2009)	**13 wk**			

2.3. Consequences of cow-calf contact on cows' performance

2.3.1. Milk yield

The frequency of milking and suckling of dairy cows influences the proportion of saleable milk (i.e. milk obtained at milking) and the total milk yield (i.e. milk yield at milking + calf milk intake) and their composition (Mendoza et al., 2010; Sandoval-Castro et al., 1999).

In the literature, evidence that suckling can increase the total milk yield, and even saleable milk output, has been reported mostly in low-producing cows in which milk ejection is difficult to activate without calf stimulation, such as Zebu×Holstein crossbred (Fröberg et al., 2007), Zebu×Brown Swiss cows (Sandoval-Castro et al., 2000) or Salers cows (Cozma et al., 2016; Cozma et al., 2013). Fröberg et al. (2007) investigated a short-time cow-calf contact system (2 x 30 min, 2h after milking) and found that adding the estimated suckled amount of milk to the milked production led to an increase of total milk production. Bar-Peled et al. (1995) found that suckled and milked Holstein cows had a higher total milk yield compared to cows milked very frequently. Also, Boonbrahm et al. (2004) found that restricted suckling (2 x 15 min after suckling) caused an increase of both saleable and total milk yield compared to not suckled cows. Other studies found an increase of total milk yield in suckled cows, but they have some limitations (Johnsen et al., 2016): Metz (1987) found an increase of total milk yield in a whole-day cow-calf contact, but only for 10 days; Margerison et al. (2002) observed it in a special breed (Lucerna breed) under tropical conditions.

However, most studies report that CCC systems reduce the amount of saleable milk (de Passillé et al., 2008; Fröberg et al., 2005; Johnsen et al., 2015b; Mendoza et al., 2010; Zipp et al., 2018; Table 1.4). The main reason is that during the suckling period, the calf's high milk intake reduces the milk yield at milking compared to not suckled cows (Johnsen et al., 2016).

However, as already reported by Fröberg et al. (2005) and Bart (2020), the reduction in milk volume collected at the milking parlour cannot be explained solely by the milk ingested by calves.

In the literature, it is reported that suckling in dairy cows selected for milk production could reduce machine-milked milk as a result of insufficient milk ejection due to suppression of oxytocin secretion (de Passillé et al., 2008; Lupoli et al., 2001).

In fact, although the effect of oxytocin release by suckling and machine milking may be comparable, when the cow is milked in the presence of the calf, suckling induces higher oxytocin release than machine milking (Tančin and Bruckmaier, 2001). The oxytocin release at milking is less marked when cows suckle their calves, either when suckling occurs just before milking (Lupoli et al., 2001) or after milking (de Passillé et al., 2008). The presence of milk remaining in the udder after an incomplete milking, induces a disturbance of oxytocin secretion and leads to a decrease in milk secretion (Barth, 2020), but this effect was observed only during milking and not during suckling (Tančin and Bruckmaier, 2001).

The type and duration of contact can also influence the milk yield. Lupoli et al. (2001) suggested that reducing the duration of CCC could limit the suppressive effects of suckling on milk ejection. Barth (2020) investigated the consequences of 3 different types of CCC (whole day, night-time and short time) on cow performance and found that restricted suckling induced the incomplete milk removal in the udder and decreased the milk secretion at milking, while the total milk yield of cows with night-time contact was not affected. These results, however, are in contrast with the findings of Johnsen er al. (2015b) who studied night-time cow-calf contact (ad libitum) and found a decrease in total milk yield and of Boden & Leaver (1994) who studied a day-time cow-calf contact (7/8 h after morning milking) and also found a reduction in total milk production.

There are also studies that found that the total milk production was unaffected by suckling: Cozma et al. (2013) found no difference in machine milk yield between cows milked in the presence of the calf

and not suckled cows, and also Kišac et al. (2011) found non-significant results comparing suckling ad libitum during 7, 14 or 21 days.
Finally, considering the highly contrasted results reported previously, it is not possible to conclude if CCC has a mainly positive or negative impact on the total milk yield. Meagher et al. (2019) reviewed the available literature and found no evidence of a negative effect of cow–calf contact on milk production over a longer period. They explain that the reduction in saleable milk during the suckling period is due to the milk consumed by the calves and it cannot be considered a loss as long as the reduction in saleable milk is lower than the amount of milk required to feed the calves. Thus, further studies are needed to evaluate the influence of suckling on lactation yield and the conditions that ensure better milk yield at milking (Johnsen et al., 2016).

2.3.2. Milk composition

Suckling could have also effects on milked milk composition, it may affect milk fat content, because the calf takes only a portion of the milk produced, and milk composition changes from low to high fat content over the course of milking (Tesorero et al., 2001).
In the literature, it is reported that usually milk fat content decreases when calves suckle their dam after milking (Bar-Peled et al., 1995; Fröberg et al., 2007; Fröberg et al., 2005; Margerison et al., 2002; Mendoza et al., 2010; see Table 1.4) and may increase when they suckle before milking (Tesorero et al., 2001). When suckling occurs after milking, the calves mainly consume the residual milk, which has a high fat content, whereas when suckling occurs before milking, the calves suckle the cisternal milk, which has a low fat content (Fröberg et al., 2007; Tesorero et al., 2001).
Moreover, the decreased fat content is an evidence for the disturbed milk ejection during machine milking of suckling cows, as oxytocin is also responsible for the secretion of fat globules (Barth, 2020; Johnsen et al., 2016). These variations in turn affect the fat content of the milked milk.
Milk protein content appears to be less affected by suckling (Bar-Peled et al., 1995; Fröberg et al., 2007; Fröberg et al., 2005; Kišac et al., 2011; Mendoza et al., 2010; see Table 1.4) as milk protein content does not change during milking. However, few studies found an increase of milk protein content in the milked milk (Barth, 2020; Boden & Leaver, 1994; Margerison et al., 2002). Milk protein content increases when the energy balance of the cows is higher (Coulon & Rémond, 1991). If suckling cows produce less total (suckled + milked) milk and presumably have similar feed intake, higher milk protein content may mean that their energy balance is probably higher than that of not suckled cows. Anyway, further studies are needed to investigate a possible effect of suckling of milk protein content.

2.3.3. Udder health

Suckling is thought to improve udder health (Fröberg et al., 2005; Margerison et al., 2002).
Several studies reported that suckling decreases the milk somatic cell counts during the suckling period and this can be related to a lower mastitis incidence (Krohn, 2001). Boden & Leaver (1994) found a significant reduction in milk somatic cell count in cows milked in the morning milking and suckled twice daily by their calves. Also Margerison et al. (2002) found a reduction in mean somatic cell count in milked and suckled cows twice daily.
The results do not demonstrate, however, that suckling and manipulation of the udder and the teats by the calf improve the udder health, but most studies suggest it (Krohn, 2001). In fact, many suggest that the reason why suckling reduces milk somatic cell count is because the calf removes residual milk from the udder, reducing the risk of bacterial and pathogenic proliferation in the residual milk, thus reducing the risk of mastitis (Beaver et al., 2019; Johnsen et al., 2016; Margerison et al., 2002). However, this hypothesis has not been proven yet.
As mentioned by De Oliveira et al. (2020), there is a lack of information on normal somatic cell count variation in cow-calf contact systems. Indeed, they reported that somatic cell count increases during

milking or suckling, so it would be important to specify, in studies that investigate on the udder health, the time and the milk fraction sampled to prevent that suckling just before sampling could bias the results (De Oliveira et al., 2020).
In the reviews of Beaver et al. (2019) and Johnsen et al. (2016) on the effects of suckling on udder health, authors concluded that the udder health of suckled cows is similar or better than in non-suckled cows (see also Table 1.4). This trend of suckling to reduce the risk of mastitis is a very important result, because mastitis is one of the most common diseases in dairy systems, and thus it could be an important argument to increase the use of some type of restricted suckling system to reduce the economic impact in farms that suffer of this disease (Beaver et al., 2019).

Table 1.4. Positive (+), negative (-) and similar (=) consequences of different CCC on cows' performance before and after the suckling period, compared to artificial rearing systems.

Authors	**Year**	**Breed**	**Type of contact**	**Duration of the contact**	**Treatment suckling**	**Suckling period**	**Milk yield during suckling**	**Milk after Separation /Weaning**	**Oxytocin**	**Milk fat content**	**Milk protein content**	**Udder health**
Bar-peled et al.	1995	Ho	Full	Short-time	3 x 15 min, 2-3 h af 3 milkings	6 wk	+	-	- during milking + during suckling	-	=	=
Barth et al.	2020	German Ho and German Red Pied	Full	Whole day Night-time Short time	Ad lib 14h 2 x 15 min before milking	100 d	- = -	- = -		-	+	+
Boden and Leaver et al.	1994	Ho	Full	Day-time then Short-time	7/8h af morning milking, then 2 x 30 min/d	8 mths	-			-	+	+
Boonbrahm et al.	2004	Ho x	Full	Short-time	2 x 15 min af milking	84 d	+	+				+
Cozma et al.	2013	Ho and Salers	Full	Short-time	2 x /d ad lib af milking + 1 min before milking	42 d	=			-	-	
de Passillé et al.	2008	Ho	Full	Short-time	2 x /d ad lib, 2h af milking	9 wk	-	=	=			
Flower & Weary	2001	Ho	Full	Whole day	Ad lib	2 wk	-	+				
Fröberg et al.	2005	Ho	Full	Short-time	3 x 2h af milking	9 wk	-			-	=	+
Fröberg et al.	2007	Ho-Zebu x Simmental	Full	Short-time	2 x 30 min, 2h af milking Ad lib + AF during the day	16 wk	+			-	=	+
Fröberg et al.	2008	Ho	Full	Short-time	2 x 30 , 2h af milkings	8 wk						=
Johnsen et al.	2015 b	Ho	Full	Night-time	Ad lib	6 wk	-					
Kišac et al.	2011	Ho	Full	Whole day	Ad lib	7, 14, 21 d	=	=		=	=	
Margerison et al.	2002	Lucerna (HFx)	Full	Short-time	2 x 15 min af milking	184 d	+	=		-	+	+
Mendoza et al.	2010	Ho	Full	Short-time	2 x 30 min, 2 h af milking	8 wk	-	=		-	=	=
Metz et al.	1987	Polish B&W	Full	Whole day	Ad lib	10 d Sep ; 60 d Wean	+	-		-		
Teeluck et al.	1981	Creole and	Full	Short-time	2 x 30 min, 30 min af milking for 30 d; then 1 x/d	90 d	+					
Zipp et al.	2018	German Ho	Full	Whole day	Ad lib	2^{nd} mth of lact	-			-		=

Table legend: Ho= Holstein; B&W= Black and White; Af= After; Mth= month; Lact= lactation; Sep= Separation; Wean= weaning; Ad lib= Ad libitum

3. Cow-calf contact and weaning distress

For most mammals, weaning is a gradual transition from the dependence on the dam and on the milk she supplies, to a social and nutritional independence (Weary et al., 2008). Under natural condition, this process involves a progressive reduction in milk intake accompanied by an increase of solid food and a gradual reduction in maternal-filial bond (Martin, 1984).
The beginning of natural weaning process seems to be connected with the age and the size of the young (Enríquez et al., 2011). For most ungulates, the weight expected at weaning seems to be equivalent to four times the weight at birth (Lee at al., 1991) while the age can vary between 7 and 14 months of age (Reinhardt & Reinhardt, 1981).
In contrast, weaning of calves in conventional systems is usually abrupt and earlier than in the natural process.
Because they are weaned at a younger age than in the wild, calves often have to cope with additional stressors such as changing social groups and environmental, i.e. mixing with unfamiliar calves in an unfamiliar pen or barn (Weary et al., 2008).
In beef extensive systems, calves are kept with their dam until 6 months of age, and weaning involves both abrupt separation from the dam and abrupt elimination of the milk diet. In dairy system instead, calves are abruptly separated from their dam at birth and they are weaned from milk diet at around 8-12 weeks of age.
Therefore, one of the critical points of dairy cow-calf contact systems is the later separation from the dam or weaning at the same time as the dam separation, because it is considered more stressful for both cow and calf than early separation (Flower & Weary, 2001).
The behavioral response to later separation can last several days (Enríquez et al., 2011), during which cows and calves vocalize, show increased activity and reduced play behavior (Fröberg et al., 2011; Lidfors, 1996; Veissier et al., 1989a).

3.1. Consequences of weaning distress on calves' behavior and growth

When abruptly and permanently separated from their dam at birth, the calves need to be accustomed quickly from a milk diet and maternal care to a solid diet, a new housing and social environment, and these changes may be manifested in behavioral signs that suggest a negative impact on the young (Newberry & Swanson, 2008). The behavioral response generally peaks during the first or second day after weaning, but under certain conditions the calf may continue to show signs of distress, such as high activity levels and high frequency of vocalizations for several days after weaning (Weary et al., 2008).

High frequency of vocalizations is one of the main indicator of stress among behavioral changes at weaning (Newberry & Swanson, 2008; Veissier et al., 1989a; Weary et al., 2008). The calf's vocalizations are interpreted as the request for maternal care (Newberry & Swanson, 2008) and the need to reunite with the dam at weaning (Weary et al., 2008). Vocalizations are also considered an evident sign of emotional and psychological distress. In the wild, vocalizations would increase the risk of attracting predators and are energy demanding (Enríquez et al., 2011; Weary et al., 2008). Another sign of distress is the increase of general activities compared to pre-weaning time budgets. Weaned calves in fact generally increase their time spent walking (Fröberg et al., 2011; Haley et al., 2005; Loberg et al., 2008), pacing (Price et al., 2003) and standing (Veissier et al., 1989) compared to time resting.

In dairy systems, weaning is a stressful event also for artificially reared calves, but diet changes alone, independently from other stressful events, have less effect on behavioral changes in the young (Weary et al., 2008). In Haley et al.'s (2005) study, beef calves showed no behavioral response when they were prevented from suckling while continuing to be in contact with their dam, while they found a strong response when they were both separated and prevented from suckling. Similarly, Jasper et al. (2008) showed a low behavioral response of artificially reared calves that had their diets changed to hot water only, demonstrating that the response to weaning appears to be related not only to nutritional factors, but primarily to other stressors at weaning, such as the separation from the milk feeding routine.

Some studies have also investigated the physiological responses of calves at weaning. Lay et al. (1998) found and increase of plasma cortisol in beef calves abruptly weaned at 6 months of age, while Veissier et al. (2013) found higher levels of blood cortisol at weaning in suckling calves compared to artificially reared ones, and finally Loberg et al. (2008) found an increase of plasma cortisol and heart rate in calves separated from foster cows at 3 months if age.

Johnsen et al. (2015c), on the other hand, quantitatively evaluated weaning vocalizations (distinguishing them into low-pitched vocalizations and high-pitched vocalizations) and concluded that high-pitched vocalizations can be a valid tool to evaluate the weaning distress. In addition, they suggested that high-pitched vocalizations are performed when calves cannot find their dam: the motivation of calf to be reunited with its dam goes beyond nutritional need since calves were still being fed milk after separation. Also in other species, vocalizations may be representative of stress at weaning: Weary & Fraser (1997) confirmed that vocalizations at weaning of piglets are an indicator of the adaptation in the post-weaning period and they found that weaning is more difficult for younger piglets (2 weeks vs 4 weeks of age). Similarly, in foals (Merkies et al., 2016) and goats (von Walter et al., 2021) the increase in vocalizations at weaning is one of the indicators of stress response.

Finally, an increase in behavioral responses was also reported in studies in which separation of cows and calves was done within a few days or a few weeks after birth. Lidfors (1996) and Weary & Chua (2000) investigated the effects of a separation after 4 days and found greater vocalizations and increased activity compared to a separation immediately after birth in both cows and calves, and Flower & Weary (2001) found similar results with a separation after 2 weeks of age.

The stress experienced at weaning may also affect growth. Changes in diet, environment and social interactions may affect the food intake of young animals and could result in reduced weight gain in the days following weaning. Moreover, this reduction could be more pronounced if the calves during the milk feeding period were not used to ingest solid food. Suckling calves often gain more weight during the suckling period compared to artificially reared calves (Fröberg et al., 2011; Mendoza et al., 2010; Roth et al., 2009; see Table 1.3), and this is mainly due to the higher milk intake. However, once weaned, it is common for suckling calves to gain significantly less weight than artificially reared ones (de Passillé et al., 2008; Froberg et al., 2001; Veissier et al., 2013; Table 1.5) and thus possibly due to lower solid food intake (Jasper & Weary, 2002). Findings in literature show that higher intakes of solid food with reduce reliance on milk, could reduce the response to weaning in term of behavioral response and weight gain (Fröberg et al., 2011; Jasper et al., 2008; Krohn, 2001; Weary et al., 2008).

In conclusion, these pronounced behavioral and physiological responses show an obvious welfare concern, and finding alternatives to reduce weaning distress could be an important production challenge for the livestock industry (Weary et al., 2008).

3.2. Consequences of weaning distress on cows' behavior and milk yield

Early separation or abrupt weaning is a cause of stress also for the cow (Haley et al., 2005). Separation a few hours after birth is considered the less stressful, but it has been shown that just a 5 min contact is enough to create a bond between cow and calf and thus cause stress to the animals at the time of separation (Hudson & Mullord, 1977). It has been reported that already a delayed separation at 4 days or a few weeks after birth increases the reaction of the cows in terms of vocalizations and increased general activities (Flower & Weary, 2001; Lidfors, 1996; Weary & Chua, 2000; Table 1.5). Therefore, when cows are separated from their calves after a long period, i.e. until weaning of the calves, it is possible that they show a higher behavioral and physiological response (Flower & Weary, 2001; Weary & Chua, 2000). There are few studies on the impact of weaning on cows. Johnsen et al. (2015c) and Veissier et al. (2013) showed that abrupt weaning induced vocalizations and agitation in the cows.

Another sign of stress for the cows could be the drop of milk at the milking machine just after weaning (Bar-Peled et al., 1995; Barth, 2020; Metz, 1987). Barth (2020) reported that cows suckling their calves before milking produced significantly less milk than not suckled cows after weaning. Also, de Passillé et al. (2008) found a drop in milk yield after weaning that returned to control levels one weeks after weaning. This could be explained by the disturbed effect of suckling on oxytocin release that makes it harder for cows to get used to the parlour when suckling ends. Anyway, this loss of milk lasts generally few days after weaning (Metz, 1987).

3.3. Ways to reduce weaning distress

As stated before, weaning in conventional systems imposes several stressors simultaneously, such as suppression of the milk diet, change in environmental and social interactions, and separation from the dam. All these factors negatively affect animals, and the combination of these intensifies their stress response. A solution to reduce the response to weaning would be to separate or modify these factors. Different procedures can help to reduce the response to weaning and can be resumed in: prevent the access to milk while the calf is still with the cow or prevent the access to the cow while the calf still has access to milk (Weary et al., 2008). Examples include practice procedures that allow cow-calf contact but prevent the calf to suckle, such as fence-line systems or anti-suckling devices (nose-flap or udder nets) (Figure 1.3). Weary et al. (2008) suggested that allowing calves to achieve nutritional independence, by preventing them from suckling before weaning, could reduce the stress at the separation from the dam. They also suggested that in order to make the solid diet more attractive after weaning one should increase the intake of solid feed before weaning, increase the weaning age and increase the separation time during the day from the dam. Johnsen et al. (2015a) showed that accustoming calves to drink from an additional milk feeder during the suckling period reduces weaning weight losses, and confirms that nutritional independence can reduce the response at weaning.

Haley et al. (2005) and Loberg et al. (2008) investigated on two-step weaning practices with nose-flaps to prevent suckling when calves and cows were still together, and found that calves weaned in two stages vocalize less, walk less, and spend more time eating and resting after separation than calves weaned conventionally. Studies in other species reported that gradual weaning can reduce the stress response. Lansade et al. (2018) compared a abruptly weaning to a progressive weaning (with fence-line) in foals and concluded that progressively weaned foals had a lower behavioral response (less neighing and trotting) but also lower cortisol levels than abruptly weaned foals.

Figure 1.3. Calf equipped with nose-flap at INRAE 'Herbipole' experimental farm (Marcenat, Fr).

Fence-line weaning is also an effective method for reducing behavioral responses to weaning (Johnsen et al., 2015c) and minimizing losses in weight gain in the days following the separation (Price et al., 2003). In addition, providing more milk during the step of separation from the dam can reduce the distress response associated with calf hunger (Johnsen et al., 2018; Thomas et al., 2001).
Finally, to accustom calves to separation from their mothers, it may be important for calves to have opportunities to experience brief separation (Weary et al., 2008). Johnsen et al. (2016) reported that half-day contact could reduce this factor because animals get used to being separated. Also Veissier et al. (2013) found that calves that were used to be separated from their dam during the night showed less behavioral response after weaning than calves that were used to be in contact with their dam during the whole day. Lansade et al. (2018) reported that training foals for weaning by accustoming them to separation from their dams can reduce the stress induced by definitive weaning, both in terms of behavioral and physiological response in foals and their dams. In addition, repeated habituation to separation from the mother can lead to stress resistance, which can help foals cope better with new situations (Lyons et al., 2010).

Table 1.5. Positive (+), negative (-) and similar (=) consequences of weaning/separation distress on calves and cows' behavior and performances.

			CALVES			COWS		
Authors	Duration of contact	Age at separation/weaning	Behavior	Concentrate intake	Growth	Behavior	Milk yield after separation/ weaning	Milk yield of lactation
Bar-Peled et al. (1995)	Short-time	6 wk					-	
Bar-Peled et al. (1997)	Short-time	6 wk	-		-			
Barth et al. (2020)	Short-time	13 wk					-	
Barth et al. (2020)	Whole day	13 wk					-	
Barth et al. (2020)	Night-time	13 wk					=	
de Passillé et al. (2008)	Short-time	8 wk			-			=
Flower & Weary (2001)	Whole day	2 wk	-		+	-		=
Fröberg et al. (2011)	Whole day	8 wk	-	-	-			
Fröberg et al. (2005)	Short-time	8 wk		-	+			=
Fröberg et al. (2007)	Short-time	16 wk		-				
Hepola et al. (2007)	Short-time	8/5 wk		+	-			
Johnsen et al. (2015a)	Day/night-time	6 wk		+	+			
Johnsen et al. (2015c)	Whole day	8 wk	-			-		
Kišac et al. (2001)	Whole day	3 wk			+		=	
Lidfors et al. (1996)	Whole day	4 d	-			-		
Margerison et al. (2002)	Short-time	26 wk					=	
Mendoza et al. (2010)	Short-time	8 wk					=	
Metz et al. (1987)	Whole day	9 wk					-	
Roth et al. (2009)	Whole day	13 wk		-	-			
Veissier et al. (2013)	Whole day	10 wk	-	-	-	-		

4. Grazing in dairy systems

4.1. Grazing dairy calves: current practices

Dairy cattle are grazing animals, so pasture is their natural environment. Access to pasture allows animal to express natural behaviors, and may reduce incidences of lameness and claw disorders (Hernandez-Mendo et al., 2007; Olmos et al., 2009). Grazing systems also reduce management and feeding costs for the farmer (Hanson et al. , 2013; White et al., 2002).
Nevertheless, the increase in productivity and the reduction of the rusticity of dairy breeds, could make pasture insufficient to meet the nutritional needs of animals and can therefore reduce the level of welfare (Charlton et al., 2011). It has also been reported that dairy cows produce 19% less milk when grazing than when confined-housing (Fontaneli et al., 2005). However, despite the lower milk production, economic models and farm surveys show that pasture-based systems may have lower operating costs and higher net income per cow compared to confined systems (White et al., 2002). Experienced grazing cattle can feed on grass as primary source and lead to reduced feeding costs (Arrazola et al., 2020).
In Le Cozler's study (2012), 60 % of dairy farms use seasonal batch calving during autumn and winter, in order to turn out animals to pasture in the following spring. Then, calves and heifers usually graze from spring to autumn, before their first year of age, but only 2% of dairy farms turn out calves to pasture before 6 months of age (Le Cozler et al., 2012). This strategy allows the synchronization of the peak of herbage growth and the peak of lactation of dairy cows, with fresh herbage covering a large part of their nutritional requirements (Ramsbottom et al., 2015). At the same time, calves have also grown and matured sufficiently and are able to be moved to pasture.
Getting young calves used to grazing will improve their welfare when they are put on pasture as adults. Charlton et al. (2017) reported that when animals are given the choice between to be on pasture or to be confined, this choice depends on several factors, such as time of day, season and where food is allocated. In addition, previous experience strongly influences this choice: the longer calves and heifers are reared with no grazing experience, the more they will choose confined-housing (Charlton et al., 2011; Charlton et al., 2017).
Inexperience can be a cause of stress and can reduce the welfare of the animal: the fear and the stress of new foods and environments may cause reduced ingestion and affect milk production (Provenza, 2002). To minimize these effects, cows should be gradually accustomed to grazing and/or calves should be put on pasture early to develop their foraging skills (Provenza, 2002). Indeed, ruminants develop foraging skills at a young age, and if they are exposed to grazing early they may graze more efficiently than animals that have not been exposed to grazing (Provenza, 1995).

4.2. Grazing dairy calves: the importance of social models

First-time grazers have to face several challenges, such as learning to eat a novel type of feed (Costa et al., 2014; Hessle, 2009), getting used to a new environment (De Paula Vieira et al., 2012b) and often coping with new individuals (De Paula Vieira et al., 2010) as heifers are often mixed when put on pasture.
As already discussed, in dairy systems heifers are raised separately from their dams and are often grouped with other heifers from the same age that have no previous experience during the first grazing season (Costa et al., 2016).
One solution to cope with the challenges faced by first-time grazers could be to provide experienced animals so that they can act as social models (Costa et al., 2016). Young animals learn by emulation of social models or by trial and error, although in most cases this last is less efficient (Thorhallsdottir et al., 1990). The social learning theory states that the best social models are the nurturers, such as the

dam, and respected peers (Bandura 1977). Indeed, naïve grazer animals foraging with social models usually ingest more forage and spend less time exploring the environment, are less vulnerable to predator attacks and malnutrition, and ingest fewer toxic plants than naïve grazer animals alone (Launchbaugh & Howery, 2005).

There are evidences that early social experiences influences calf response to novelty (Costa et al., 2014; De Paula Vieira et al., 2012a). It is important to know that ruminants face to novel food tend to taste it in small quantities or to reject it, this behavior is called neophobia, that means "fear of new". It is a survival mechanism that limits the consumption of toxic plants (Launchbaugh et al., 1997). Therefore, calves usually are neophobic: they tend to choose feed and places they already know, so the individual learning in a new environment takes more time than learning by social models (Costa et al., 2014; Launchbaugh et al., 1997).

The dam is the primary social model and plays an important role in the acquisition of foraging behavior and feed selection (Hessle, 2009; Pullin et al., 2017). At birth it is the dam who influences the calf's ability to find the teat and to suckle efficiently, while at weaning it is always the dam who gradually prevents the calf from suckle, stimulating it to seek alternative feeds (De Paula Vieira et al., 2012; Reinhardt & Reinhardt, 1981). In addition, the presence of adults during the first few weeks of life stimulates calves to sample small amounts of solid feed (Key and Maciver, 1980; Nolte et al., 1990). The presence of the dam has been shown to encourage calves to prefer a new diet for at least 12 weeks after the first exposure (Fukasawa et al., 1999).

In addition, De Paula Vieira et al. (2012b) reported that early socialization for dairy calves during the milk feeding period, i.e. the first 6-8 weeks of life, can help to reduce the problems associated with the transitions to new feeding and social environments. Social contact with the dam and other calves could also decrease responses to restraint and increase play behaviors (Duve et al., 2012).

Letting calves to graze with their dams and conspecifics allows them to develop efficient grazing behavior through observation and imitation, and teaches them to identify nutrient plants that meet their nutritional needs and to avoid dangerous ones (Arrazola et al., 2020; Fukasawa et al., 1999; Thorhallsdottir et al., 1990). Pullin et al. (2017) found that lambs grazing with their dam spent more time foraging, were more active, developed long-term feed preferences and learned aversion to toxic feed more effectively than lambs grazing alone. Also Hessle (2009) suggested that first-grazing season calves that were put on pasture with experienced conspecifics grazed more actively than calves put on pasture with inexperienced conspecifics, and they found that the effect of the presence of conspecifics on calf grazing activity development disappeared after 1 month. Lopes et al. (2013) observed that early grazing experience of heifers only affected grazing behavior and milk production in the first days on pasture but showed that the animals would generally adapt to a new environment and a novel feed easily, especially during their first year of life.

Dairy calves that have learned to graze with their dam might therefore more efficiently recognize herbage quality and select specific patches when turned out to pasture after weaning, compared to calves that never grazed before. However, it is unclear whether this advantage holds only in the first grazing day or is more persistent.

5. Economic sustainability

If tradition was an important reason for practicing early separation of calves from their dams, economics also greatly influences this decision. If it is wanted to promote more natural rearing systems that allow cow-calf contact, these systems must be profitable, either because of reduced production costs or because consumers being willing to pay more for such production (Asheim et al., 2016). Therefore, this is for this reason that insights on the economic consequences of the implementation of CCC practices is an important topic for dairy farming.

Studies have shown that implementing a dam-calf system may require changes in daily practices and long-term priorities of farms compared to conventional systems with early separation (Vaarst et al., 2020). Besides the technical aspects, the new way of interacting and perceiving the animals can also be challenging for farmers, all these factors must be taken into account during the transition towards new innovative practices (Ivemeyer et al., 2015; Vaarst et al., 2020). The growing interest in dam-calf rearing makes it possible to investigate the application of these practices in different types of farming systems (Vaarst et al., 2020).

The consequences on animal welfare, health and production in cow-calf systems have been previously studied and discussed, and many authors pointed out certain positive aspects on animal welfare (Knierim et al., 2020). However, the economic consequences of these systems have remained poorly studied. Bickelhaupt and Verwer (2013) concluded that the incomplete economic information on this alternative farming system makes it difficult for farmers to consider a possible conversion.

The multitude of rearing practices and farm factors that influences economic analysis makes this type of study not easy to realize.

The study of Asheim et al. (2016) on the investigation of how to maximize profit on Norwegian dual purpose dairy-beef farms, showed that, under the conditions investigated, suckling for 7 weeks could be economically optimal compared to not suckling or suckling for 13 weeks.

The lack of other studies on this topic still makes unclear what is the best approach to perform this type of analysis. Indeed, it is not easy to predict economic changes on a theoretical basis in systems involving CCC, because there is a large variety of rearing systems with as many number of interaction factors, even if there are common factors among the different systems such as the reduction in the amount of saleable milk or the increased growth rate of calves.

The main factors to take into account, however, are milk production and composition, milk ingestion and calf growth, the health status of the animals but also the possible reduction of workload for the farmer. It is important also to consider that modern farm buildings today are not always designed for rearing cows and calves together (Asheim et al., 2016). Slatted floors conceived for cows could be a problem for calves. Therefore, some farmers may need to invest in additional buildings and interiors if they consider having cows and calves together in a loose-housing system.

The farm's gross operating profit can be positive if there is a reduction of costs, but if it is not profitable some kind of regulation might also be implemented to enforce the practice, for example through the creation of a label that protects and ensures this type of alternative production, that is more ethical and respectful of animals.

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OBJECTIVES

Growing public interest in livestock animals' welfare led the scientific community to question alternative rearing practices that aim to restore animal welfare and appropriate behaviors for each species. Concerning the dairy production, the most common welfare concerns are the early separation of calves from their dams and the restricted access to pasture for animals in intensive systems.
Several studies already exist in literature about alternative rearing systems for dairy calves. In last years, indeed, the interest of the scientific community for this topic has grown significantly. Most studies focused either on animals' performance or on animal welfare. However, a complete analysis of all the consequences of this practice is not available yet.
Two European CORE organic Cofund projects have been financed to support research on this topic (from 2018 to 2021): *Grazy DaiSy* which aims to promote the use of grazing for dairy cows and the rearing of calves with the cows while minimising the use of medicines, and *ProYoungStock* which aims to study the (long-term) effects of natural rearing and feeding strategies on health, welfare and economic performance. This thesis was part of the *ProYoungStock* project.
The overall aim of the thesis was therefore to study deeply and exhaustively all the aspects that a cow-calf contact system can influence in a dairy rearing system and to find the practice that is the best compromise between animal welfare, animal performance and economic impact.

The objectives of this thesis were to:

1. **Quantify the consequences on animal performance and stress at weaning of four dam-calf contact rearing systems**
   The aim was to identify ways to maintain cow-calf contacts so as to balance the benefits for calves' growth vs. the negative impacts on saleable milk and stress at weaning.

2. **Study the influence of early-life dam-calf contact and grazing experience on post-weaning behavior and herbage selection of dairy calves in the short term**
   We tested the hypothesis that different early-life experiences may influence the grazing and social behavior of calves in the short term after weaning.

3. **Find the best trade-off between societal demand and economic sustainability for farmers of different dam-calf contact rearing systems**
   We simulated the impact of different suckling systems on the economic results of intensive, extensive and organic farming systems in France.

Strategies

To achieve the objectives of the thesis, three trials were carried out at the French National Institute for Agriculture, Food and Environment (INRAE) 'Herbipôle' experimental farm (https://doi.org/10.15454/1.5572318050509348E12) located in Marcenat, France (45.30°N, 2.84°E, 1 080 m a.s.l.). All procedures were carried out in accordance with French Ministry of Agriculture guidelines on animal research and with all other applicable national and European regulations governing experiments with animals.

Trial 1 (called Volame 1) was carried out between February and July 2017, Trial 2 (Volame 2) was carried out between February and July 2018 and Trial 3 (Volame 3) was carried out between February and August 2019.

My involvement on the project started in 2018 when I came to France to realize the Erasmus exchange for 5 months as part of my master degree. Then I came back in 2019, during the first year of the PhD. I therefore participated in the drafting and in the execution of Trials 2 and 3. As part of the PhD project, I have thus only recovered the data collected during Trial 1 and used it for the analysis.

To answer the **first objective** of the thesis we compared four different cow-calf contact practices to an artificial suckling practice, i.e. where calves were separated from their dams immediately after birth and fed with an automatic milk feeder until weaning (*Control*).

In the first trial (Trial 1), we tested two practices where calves had access to dams for a short period every day until weaning (20 min '*Before*' and 2.5 h '*After*' morning milking). We aimed to study if a short-time contact could be a good compromise between milk loss at parlour and calf growth. The results showed that suckling for 2.5 h immediately after milking fails to cover the calves' needs and significantly reduces the amount of saleable milk, while suckling for 20 min before milking satisfies the calves' needs but drastically reduces the amount of saleable milk.

Considering these results, we decided to test a day-time contact that allows a longer period for calves to suckle and perhaps reduce milk loss at parlour, as calves could suckle between the two daily milkings. Therefore, in Trial 2, we tested a suckling practice where calves had a day-time access to dams until weaning (*Dam*, 9 h per day).

In both trials, all female and male calves were kept for the study.

Finally, in the third year we decided to investigate on suckling practices that were closer to current realities, i.e. in each group five male calves were sold at 4 weeks of age and cow-calf contact was allowed only during the first weeks of life, as it is usual in dairy farms that practice natural suckling in France (Michaud et al., 2018). We have also reduced the cow-calf contact time between milkings to try to reduce the losses of saleable milk. Therefore, in Trial 3, we tested two suckling practices where in the first one the calves had a day-time access to dams until weaning (*Dam*, 6 h per day) while in the other one the calves had a day-time access to dams until 4 weeks of age before being separated and reared as calves of the Control group until weaning (*Mixed*).

To answer the **second objective** of the thesis we used the three different early-life calves' experiences in Trial 3 to study their effects on the grazing and social behavior after weaning. We classified the three different backgrounds into: *Control* calves that had been separated at birth from their dam and had never experienced grazing, *Dam* calves that had been reared and grazed with their dam until weaning and Mixed calves that had been separated from their dam at 4 weeks of age and had never experienced grazing.

Finally, to answer the **third objective** of the thesis we used part of the measurements performed in the three trials to simulate the economic impact of two suckling practices (*Short-time contact* and *Day-time contact*) on three different farming systems in France, supported by a data collection device called Diapason.

The thesis is structured into 6 chapters. Chapter 1 is a general overview of the available literature on CCC practices. Chapters 2 and 3 address the first objective of the thesis by investigating the consequences of different CCC practices on animal performances, health and stress at weaning. Chapter 4 presents the study on the influence of early-life dam-calf contact and grazing experience on post-weaning calves' behavior, to answer the second objective of the thesis. Chapter 5 describes the economic analysis performed to address the third objective. Ultimately, the objectives, results of and perspectives from the research carried out in the present thesis are discussed in Chapter 6.

CHAPTER 2

Animal performance and stress at weaning when dairy cows suckle their calves for short versus long daily durations

This chapter is based on: [1,2]Nicolao, A., [1]Veissier, I., [3]Bouchon, M., [2]Sturaro, E., [1]Martin, B. and [1]Pomiès, D. Animal performance and stress at weaning when dairy cows suckle their calves for short versus long daily durations. Submitted to *Animal*, on December 27, 2021.

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Abstract

Calves in most European dairy farms are separated from their dams either immediately or within a few hours after birth, prompting increasing society concern on animal welfare reasons. The aim of this study was to identify practices to maintain cow–calf contact (**CCC**) that balance the benefits for calf growth and health against the negative impacts on sellable milk and stress at weaning. We tested reuniting cows and calves for 20 min before (**Before**-group) or 2.5 h after (**After-**group) morning milking (Trial 1) or for a 9 h period between the morning and evening milkings (**Dam**-group, Trial 2). **Control**-calves were separated from their dam at birth and artificially suckled with tank milk provided daily at 13% (Trial 1) and 14% (Trial 2) of their body weight. In both trials, each practice was applied on a group of 14 dam–calf pairs (7 Holstein [**Ho**] and 7 Montbéliarde [**Mo**]). All calves were weaned at a weight of at least 100 kg. In Trial 1, the After-group was prematurely stopped when the calves were 8 weeks of age as calf growth became limited (318 g/d) due to low milk intakes (2.93 kg/d). During the first 8 weeks of lactation, milk yield at the parlour was 29%, 51% and 42% lower in After-, Before- and Dam-cows respectively compared to Controls. From Week 14 to 16 when all calves were separated from their dam, Before-cows still produced 25% less milk than Control-cows while Dam-cows reached the milk yield of Control-cows within a week. There were no significant differences in milk Somatic Cells Counts (**SCC**) and in frequency of cow and calf health events between suckling and control groups. Compared to Control-calves, calf growth until weaning was higher in the suckling calves in Trial 1 (873 vs. 702 g/d) and similar in Trial 2 (935 vs. 928 g/d). At weaning, Before- and Dam-calves started to vocalize earlier and continued to vocalize longer than Controls. In conclusion, the best compromise between cow milk yield and calf growth is a long period of CCC (9 h) between the morning and evening milkings. Still abrupt weaning stresses both cows and calves even if CCC has been restricted before separation.

Keywords: cow-calf contact, milk feeding, milk yield, growth, weaning

Implications

Consumers are increasingly questioning the practice of separating calves from their dams at birth. Compared to short periods of suckling immediately before or after milking, a long period of suckling between milkings provides a good compromise between sellable milk and calf growth. This practice could conciliate consumers and farmers views. Solutions should be explored to reduce calves and cows stress due to abrupt weaning.

Introduction

Calves in most European dairy farms are separated from their dams either immediately or within a few hours after birth (Busch et al., 2017). They are fed milk replacers or non-marketable milk, distributed by an automatic milk feeder or in buckets, and receive increasing amounts of solid feed until weaning (Le Cozler et al., 2012). Female calves, which are the future replacement heifers, are generally weaned off from milk at 8–10 weeks of life, or 12 weeks of age or more in case of organic farming. Male calves are generally sold at a few weeks of age to be fattened in specialized farms. Dairy calves therefore very rarely suckle their dam (Pardon et al., 2012). Early separation between cows and calves enables farmers to get the most milk from the cows and control colostrum and milk ingestion by the calves. This practice however creates animal welfare issues for consumers (Agenäs et al., 2017) who associate the welfare of an animal with possibilities to express its natural behaviors (Lund et al., 2006). In dairy farming, this includes calves suckling their dam. Some farmers already let calves suckle their dam or other cows at least for short periods after birth, either to promote calf health or to reduce workload and/or production costs (Michaud et al., 2018).

Several experiments were carried out to assess how letting dairy cows suckle affects milk production and composition. Considering the total milk produced by cows (either milked or taken by the calves), a combination of milking and suckling can make cows produce more milk due to teat stimulation by the calves and better udder emptying (Sandoval-Castro et al., 2000). This increase in total milk production — and even sellable milk output — was reported in dual purpose breeds, such Salers cows, in which calf stimulation before milking is necessary to activate milk ejection (Cozma et al., 2013; Cozma et al., 2016).

However, in dairy cows selected for high milk production, total milk production is sometimes reported to be unaffected by nursing (Mendoza et al, 2010) in particular when sucking occurs just before and after milking (Cozma et al., 2013). Most of the time, free cow-calf contact (**CCC**) is found to significantly reduce the milk yield by 10 to 12 kg/d (Pomiès et al., 2010; Zipp et al., 2018). Krohn et al. (2001) suggested that reducing the duration of CCC could limit the suppressive effects of suckling on milk ejection. Suckling may also affect milk fat content, because the calf takes only a portion of the milk produced, and milk composition changes from low to high fat content over the course of milking (Tesorero et al., 2001). Consequences of suckling on milk protein content are not clear so far (Johnsen 2016) : Boden and Leaver (1994) and Barth (2020) found an increase of milk protein content in suckled cows while Cozma (2013) found a decrease, and Fröberg (2017) and Sandoval-Casto (1999) found no differences.

Calves reared with their dam can grow faster compared to artificially reared calves, in particular when the latter are fed a restricted milk diet (Flower & Weary, 2001). The faster growth may be due to higher milk consumption as well as to stimulation of anabolism by the higher release of oxytocin in suckling calves than bucket-fed calves (discussed by Uvnäs-Moberg et al., 2001). Therefore, suckling may have positive effects on calves' growth that are not solely related to the higher ingestion of milk.

A common belief is that separating the calf at birth limits the risk of transmission of diseases and is better for both calf and cow health. Beaver et al. (2019) reviewed the available literature and found no evidence that suckling has negative effects on health. For instance, when latency to first suckling and quality of colostrum are controlled, leaving the calf with its dam at birth has no effect on risk for diarrhoea or on mortality (Meagher et al., 2019). In addition, suckling can benefit cows by reducing the incidence of mastitis (Johnsen et al., 2016), and Pomiès (2010) and Cozma et al. (2013) reported lower milk SCC in nursing cows.

Under natural conditions, weaning occurs gradually at 8–10 months of age (Reinhardt & Reinhardt, 1981) , and the cow–calf bond persists after weaning (Veissier et al., 1990). In dairy farming promoting CCC over long duration, weaning is provoked by an abrupt separation of dam and calf that causes stress to both cows and calves (Flower & Weary, 2001; Hudson & Mullord, 1977; Lidfors, 1996; Weary &

Chua, 2000). Cow–calf bonding is viewed as positive for animal welfare, but the stress induced by the separation is a welfare problem (Weary et al., 2008). Habituating calves to be separated from their dam can reduce stress at weaning (Price et al., 2003).

Restricted suckling, i.e. suckling for only some time during the day, is likely to be beneficial to calf growth and health while limiting losses in total and sellable milk production and limiting stress at weaning. The aim of this study was to propose suckling practices that achieve these balanced effects. We tested reuniting cows and calves for short periods before or after milking or for a longer period between the morning and evening milkings. Reuniting cows and calves was generally performed only once a day with a view to limit farmers' labour while still allowing adequate calf growth (Ackerman et al., 1969; Saldana et al., 2019).

Material and methods

We conducted two trials at the French National Institute for Agriculture, Food and Environment (INRAE) 'Herbipôle' experimental farm (https://doi.org/10.15454/1.5572318050509348E12) located in Marcenat, France (45.30°N, 2.84°E, 1 080 m a.s.l.). All procedures were carried out in accordance with French Ministry of Agriculture guidelines on animal research and with all other applicable national and European regulations governing experiments with animals. All researchers responsible for the study (D. Pomiès, B. Martin, M. Bouchon, and I. Veissier) and all animal caretakers had adequate appropriate training, and the experimental farm is accredited for running experiments (C15-114-01).

Trial 1 was carried out between February and July 2017, and Trial 2 was carried out between February and July 2018. In Trial 1, two practices where calves had access to short-time CCC every day ('**Before'** and **'After'** milking) were compared to artificial suckling practice (**Control**). In Trial 2, a suckling practice where calves had access to day-time CCC every day (**Dam**) was compared to artificial suckling practice (**Control**). Each practice was applied on a group of 14 dam–calf pairs (7 Holstein [**Ho**] and 7 Montbéliarde [**Mo**]), called with the same name (Before-, After-, Dam-, or Control-group). Within each trial, the 14 dam–calf pairs were balanced for lactation number (2.55 ± 1.68 in Trial 1, 2.71 ± 1.56 in Trial 2), date of calving (28 March ± 22 d in Trial 1, 17 March ± 14 d in Trial 2) and milk yield genetic index (84 ± 294 in Trial 1, 106 ± 205 in Trial 2). The groups were decided before calving; the sex of calves was therefore not balanced. In Trial 1, 78%, 29% and 50% of the calves were female in Before-, After- and Control-groups respectively and in Trial 2, 36% and 29% of the calves were female in the Dam- and the Control-group respectively. Cows were milked in a 2 x 14 herringbone milking parlour (Delaval, France) twice a day at 07:00 am and 04:30 pm.

Animal management

Before weaning in Trial 1. The Control-calves were separated from their dams within a few hours (up to 6 h) after birth. They received at least 2.0 L of fresh colostrum from a feeding bottle. If there was no good-quality fresh colostrum available (< 24% Brix, measured by refractometer), then good-quality thawed and reheated colostrum was provided. The calves were housed in individual pens for 7–9 days and fed bulk milk twice a day using buckets equipped with teats, then accommodated in a collective straw-bedded pen of 60 m² with access to water and a hay rack until weaning. They were fed bulk milk by an automatic feeder equipped with teats (Förster Technik Engen, Germany), specific starter-age concentrate (Startivo, Centraliment, 15006 Aurillac) distributed in a bowl within the same automatic feeder as for milk, and hay (permanent grassland, first cut). Amount of milk provided to the calves was about 13% of their body weight (BW) (12% to 15% depending on the week) during the first seven weeks of age, then decreased from 10% to 3% BW from Week 8 to Week 12 to prepare the calves for weaning (Table 1). In the two remaining groups, the calves spent the first three days after birth with their dams in a 20 m² individual calving pen or a 40 m² collective pen for three cows and their calves, depending on pen availability. All pens were equipped with water troughs. During these days, animal caretakers checked at least twice a day if the calves suckled properly their dam. Then calves were separated and accommodated in a collective pen until weaning and cows returned to the herd. Before-calves had access to their dam in a specific collective pen facing the milking parlour (90 m²) for 20 min before the morning milking and 10 min before the evening milking for the first two weeks, then only for 20 min before the morning milking. After-calves had access to their dam in a cow pen for 2.5 h after the morning and evening milkings for the first two weeks, then only for 2.5 h after the morning milking. The calves were also fed concentrate by an automatic feeder. The calf feeding plan is detailed in Table 1.

Before weaning in Trial 2. Control-calves were reared in the same way as those in Trial 1, except that the amount of milk available in the automatic milk dispenser was 14% of calf BW during the first seven weeks then decreased from 8% BW in Week 8 to 3% BW from week 10 (Table 1). Because there were less calves to be suckled by their dam in Trial 2 than in Trial 1, Dam-calves spent the first five days after birth (and not only the first three days as in Trial 1) alone with their dam in a straw-bedded 20 m² individual calving pen (and never in a collective calving pen as sometimes done in Trial 1). The calves received colostrum directly from their dam. The calves were then housed in a collective straw-bedded pen next to the cowshed (50 m²), from which they could see their dams. From 07:30 am when cows came back from the milking parlour to 04:30 p.m., the separation gate between the calves' pen and the cowshed was left open so that the calves could suckle their dams. Calves had free access to water, a hay rack and a bucket with concentrate. The calf feeding plan is detailed in Table 1.

Cow feeding in Trials 1 and 2. The cows were fed ad libitum with a mixed ration (82% 1st-cut hay, 18% 2nd-cut hay in Trial 1; 40% 1st-cut hay, 60% 2nd-cut hay in Trial 2) plus 5 kg/d of concentrates (Centraliment) distributed twice a day until early May (1.5 kg/d GalaProteine 40% Crude Protein (**CP**) and 3.5 kg/d GalaCorExpe 15% CP in Trial 1; 1.0 kg/d GalaProteine and 4.0 kg/d GalaCorExpe in Trial 2). From early May, the cows went to pasture day and night, and received 2 kg of concentrate (GalaPature 15% CP) after each milking. During the two periods, cows had free access to water. In Trial 1, the three groups of cows grazed together during the daytime and all calves stayed inside the barn. In Trial 2, the two groups of cows grazed in two adjacent plots, swapping every morning to ensure the same feeding, during the daytime. Dam-calves went to pasture with the cows, while Control-calves stayed inside the barn.

Calf weaning in Trials 1 and 2. Weaning took place in batches, every two weeks. The calves were weaned at a weight of at least 100 kg, which was reached in average on Week 13 in Trial 1 (108 ± 3.9 kg) and Week 11 in Trial 2 (114 ± 2.2 kg). On the day of weaning, the calves to be weaned were moved to two collective pens [one for each group, 10 x 70 m] for one week, and then to the same collective pen (Trial 2) or were moved directly to a collective pen (Trial 1) due to lack of pen availability. After weaning calves and cow could not see each other, but they could hear each other as they were housed in the same cowshed. Post-weaning calves were fed concentrate and hay (Table 1).

Table 1. Feeding plans (milk, concentrate, hay) used for the three groups of calves in Trial 1 (‘Control’, ‘Before’, ‘After’) and the two groups of calves in Trial 2 (‘Control’ and ‘Dam’) during the first 16 weeks of age. Control-calves were separated from their dam at birth, Before-calves were able to suckle their dam for 20 min before the morning milking and 10 min before the evening milking for the first two weeks, After-calves were able to suckle their dam for 2.5 h after the morning and the evening milkings for the first two weeks, then for 2.5 h after the morning milking, and Dam-calves were able to suckle their dam for 9 h between the morning and evening milkings.

Trial	Group		Weeks of age															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	Control	Milk[1] (kg/d)	6.0	6.0	7.0	8.0	8.0	8.0	8.0	7.0	6.0	5.0	4.0	3.0	→ weaning			
		Concentrate[2] (kg/d)	0.0	0.0	0.2	0.2	0.3	0.5	0.7	0.9	1.1	1.4	1.7	2.0	~2.0	~2.0	~2.0	~2.0
		Hay[3]	0.0	0.0	ad libitum													
	Before	Suckling period[4]	Twice a day		Morning										→ weaning			
		Concentrate (kg/d)	0.0	0.0	0.2	0.2	0.3	0.5	0.7	0.9	1.1	1.4	1.7	2.0	~2.0	~2.0	~2.0	~2.0
		Hay	0.0	0.0	ad libitum													
	After	Suckling period	Twice a day		Morning										→ weaning			
		Concentrate (kg/d)	0.0	0.0	0.2	0.2	0.3	0.5	0.7	0.9	1.1	1.4	1.7	2.0	~2.0	~2.0	~2.0	~2.0
		Hay	0.0	0.0	ad libitum													
2	Control	Milk (kg/d)	6.0	7.0	9.0	10.0	10.0	10.0	9.0	7.0	5.0	3.0	3.0	3.0	→ weaning			
		Concentrate (kg/d)	0.0	0.0	0.2	0.4	0.6	0.9	1.2	1.5	1.8	2.0	2.0	2.0	~2.0	~2.0	~2.0	~2.0
		Hay	0.0	ad libitum														
	Dam	Suckling period	24/24	From morning to evening milkings											→ weaning			
		Concentrate (kg/d)	0.0	ad libitum											~2.0	~2.0	~2.0	~2.0
		Hay	0.0	ad libitum														

[1] Bulk milk distributed individually by automatic feeder
[2] Starter-age concentrate distributed individually by automatic feeder (Trial 1 and the Control group in Trial 2) or in a collective bucket (Dam-group in Trial 2)
[3] Permanent grassland hay (first cut) distributed in a rack
[4] Suckling calves spent the first 3-5 days after birth with their dam

Measurements

Trial 1. Individual milk yield at parlour was measured at each milking using milk flow meters (MM27BC, DeLaval, Tumba, Sweden). Individual milk samples of 30 mL were taken on four consecutive milkings per week and analyzed at Agrolab's (Aurillac, France) to determine milk fat and milk protein contents by mid-infrared spectroscopy. Milk somatic cell counts (**SCC**) were measured by epi-fluorescence on two consecutive milkings per week until 16 weeks after calving to calculate average individual milk yield at the parlour and milk composition by week of lactation.

Calf body weight (**BW**) was measured at birth then every Tuesday morning until 15 weeks of age to calculate individual average daily gain (**ADG**) (*ID 300* scale, TRU-TEST; 0.5 kg precision, up to 250 kg). Before- and After-calves were weighed just before and 20 min after joining the cows, and the measures served to estimate the individual milk intake by suckled calves per week until weaning as the difference in BW after and before suckling. Daily milk intake by Control-calves was recorded by the automatic milk feeder until weaning.

Animal caretakers checked clinical signs on animals at least once a day, they applied Standard Operating Procedures to cure the animal affected and recorded the disorder and the treatment used in a sanitary logbook. The quality of the records was checked once a week by one of the author of the present paper (M. Bouchon). Health disorders were sorted into reproductive disorders (metritis, retention of the placental membrane, ovarian cysts, and vaginitis) and non-reproductive disorders (mastitis, milk fever, lameness, etc.) for cows, and respiratory disorders (runny nose, coughing, dyspnoea, etc.) and non-respiratory disorders (diarrhoea, umbilical infection, etc.) for calves.

Around weaning (the day before weaning [Day 0], the day of weaning [Day 1], and Day 2, Day 4 and Day 7 after weaning), the calves and their respective dams were observed with continuous direct observations by two trained observers. Observations started at 02.00 p.m. and last for 5 min to note whether they vocalized *frequently* (seven vocalizations per min or more), *from time to time* (from one to six vocalization per min), or not (zero vocalizations), with no distinction between high or low pitched vocalizations. We then calculated the daily percentage of animals that vocalized (frequently or from time to time), by group and by type of animal (calf or cow).

Trial 2. Milk yield, milk composition, health disorders and observations around weaning were measured in the same way as for Trial 1.

Cow BW was measured at calving (on two consecutive days) then once a month and at weaning (*IRW ql* scale, DELAVAL; 1 kg precision, up to 15 000 kg). On the same dates, cow body condition score (**BCS**) was estimated on a scale of 0 (very thin cow) to 5 (very fat cow; Bazin, 1984).

Calf BW was measured at birth then every Tuesday morning until 16 weeks of age to calculate individual ADG between birth and weaning and at three weeks before and after weaning (same scale as in Trial 1). Daily intake of milk by calves was not controlled.

From the beginning of March to the start of the grazing period (beginning of May), once a week, Dam-cow/calf pairs were observed during the first hour after the morning milking by two trained observers who recorded all the calves' successful and refused suckling attempts from their dam or from other cows. To estimate the acceptance by cows of calves other than their own, we calculated the ratio between percentage of successful sucking attempts by their own calf and that by other calves. A ratio > 1 indicates that the cow accepts more her calf than another.

Statistical analyses

The data were analyzed using the MIXED procedure of the SAS 9.4 software package (SAS Institute Inc., Cary, NC). SCC were log10-transformed to achieve normal distribution. Individuals - cow or calf - were considered as statistical unit and used in the models as random factors. For milk yield, milk composition, cow BW and BCS, the model took into account the effects of practice (Control group and two suckling groups in Trial 1; Control group and one suckling group in Trial 2), breed (Ho or Mo), parity (primiparous or multiparous) and interactions group × breed and group × week (for milk yield and milk composition only) as fixed factors, week of lactation as repeated factor (for milk yield and milk composition only), and date of calving as well as initial values at calving (milk yield index, fat content index, protein content index, and BCS) as covariates. For calf BW, ADG and daily milk intake, the model took into account the effects of practice, breed, sex, and the interactions group × breed and group × week (for BW and milk intake only) as fixed factors, week of age as repeated factor (only for BW and milk intake) and date of birth and BW at birth as covariates. For all data, normality of residuals was checked using the Shapiro-Wilk test and their homogeneity was checked visually. Frequency of health disorders and vocalizations around weaning was compared between groups using a Chi-squared test. Significance was set at $P < 0.05$. The ratio acceptance of other calves by cows was compared to 1 (no difference between own calves and others) using a Wilcoxon t-test. As the weaning took place between the 9th and the 13th week, the statistical analyses were carried out successively on 2 periods: before weaning (Weeks 1-8), with post-hoc comparisons between groups in Trial 1, and after weaning (Weeks 14-16). Results are expressed as means and Standard Errors in tables and figures. In the text, averages of the differences between groups or breeds are reported.

Results

Trial 1

The experiment was stopped for the After-group on 12 June 2017, when After-calves were about 8 weeks of age (see the subsection headed *Cow and calf health for details*). The results reported in Table 1 refer to Weeks 1 to 8 for all groups and to Weeks 14 to 16 for Control- and Before-groups only.

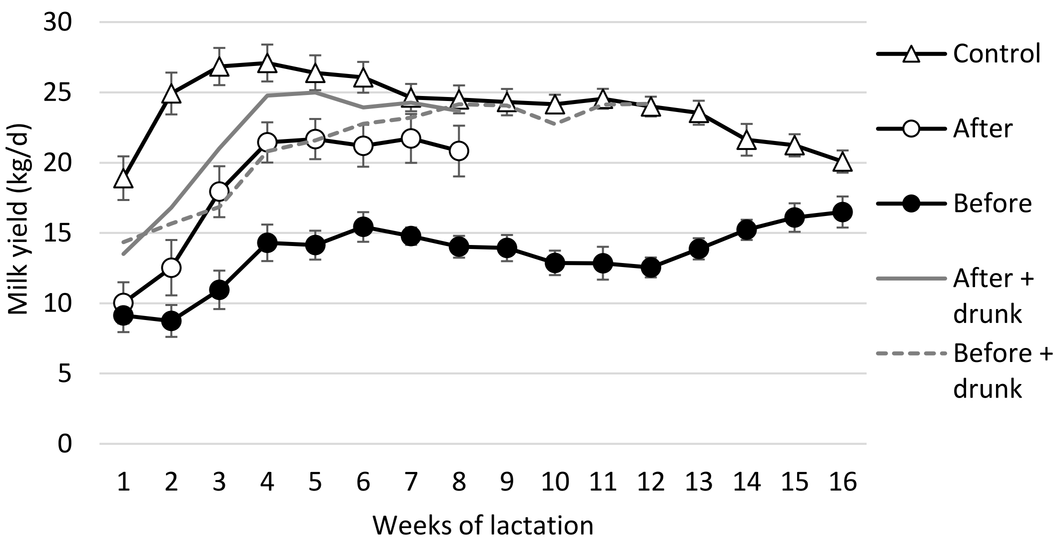

Figure 1. Daily milk yield at the parlour for the three groups of cows in Trial 1, by week of lactation (means and standard errors of raw data). In the Control-group, the calves were separated from their dam at birth; in the Before-group, the calves were able to suckle their dam for 20 min before the morning milking; in the After-group, the calves were able to suckle their dam for 2.5 h after the morning milking. Weaning took place when the calves weighted at least 100 kg. Each group consisted of 7 Holstein (Ho) and 7 Montbéliarde (Mo) cow-calf pairs. The grey lines represent the sum of the daily milk yield at parlour of cows and the milk drunk by calves (Before and After groups).

Milk yield at parlour. During the first 8 weeks of lactation, Before-cows produced 12.6 kg/d less milk per cow and After-cows produced 7.1 kg/d less milk per cow than Control-cows. The difference was marked especially at morning milking in Before-cows (-11.1 kg/d; Table 2). The difference in milk yield between Before- and Control-cows increased up to 13.1 kg/d from Week 1 to Week 4, then it stabilized at 11.0 kg/d from Week 5 to Week 12 (Figure 1). The difference in milk yield between After- and Control-cows decreased by up to 6.3 kg/d from Week 1 to Week 4, then it stabilized at 4.5 kg/d until the trial was stopped for After-cows on Week 8. Milk yield was always higher for Ho cows than Mo cows (+4.5 kg/d from Week 1 to Week 8 and +0.8 kg/d from Week 14 to Week 16). There was no interaction between breed and cow group.

The estimated total milk yield by suckling cows – including the milk suckled by calves (see below) – was lower than the milk produced by Control-cows, by 5.3 kg/d in Before-cows and 4.2 kg/d for After-cows. The difference was especially marked on Week 2, with Before- and After-cows producing 9.3 kg/d and 8.1 kg/d less than Control-cows, then it gradually faded by Week 7. From Week 14 to Week 16, milk yield of Before-cows increased but remained lower than that of Controls (-5.2 kg/d).

Milk composition at parlour. During the first eight weeks of lactation, milk fat content was higher for Before-cows (+0.32 percent point [pp]) and lower for After-cows (-0.46 pp) compared to Control-cows (Table 2). Milk fat content in morning milk was lower in Before-cows than in the other groups (+0.38 pp on average), whereas milk fat content in evening milk was lower in After-cows than in the other groups (+8.3 g/kg on average). No difference were found between breeds but a significant Group × Breed interaction was observed, with After-cows milk fat content being lower in Mo cows than in Ho cows, in both morning and evening milking (-0.49 pp, on average). Milk protein content was higher in Before-cows than in the other groups (0.29 pp on average) and this difference was similar in morning and evening milk. Milk protein content was higher in Mo cows than in Ho cows in all three groups in morning and evening milk (+0.20 pp on average). From Week 1 to Week 8, Before-cows and After-cows had over 30 000 somatic cells/mL milk more than Control-cows; the differences in SCC between groups or breeds were nevertheless non-significant. From Week 14 to Week 16, Before-cows still had about 30 000 cells/mL more than Control cows (P = 0.032).

The differences in milk composition between Before-cows and Control-cows from Week 14 to Week 16 were similar to those recorded from Week 1 to 8.

Milk intake by calves. During their first eight weeks of life, Before-calves ingested more milk (+1.26 kg/d) and After-calves ingested less milk (-3.09 kg/d) than Control-calves (Table 2), with no overall between-breed difference but a significant breed × group interaction. In Control-calves, there was no difference in milk intake between Ho and Mo, but whereas within Before-calves, Ho ingested more milk than Mo (+1.24 kg/d) within After-calves, Ho ingested less milk than Mo (-1.09 kg/d). From birth to weaning, Before-calves ingested 2.80 kg/d more milk than Control-calves. Again, in Control-calves, no difference between breed was observed but within Before-calves, Ho ingested more milk than Mo (+1.10 kg/d).

Calf growth. From Week 1 to Week 8, After-calves had 50% less ADG than other calves (Table 2). The BW difference between Control-calves and After-calves increased progressively from 2.5 kg to 13.9 kg (Figure 2). Because of this low growth and a number of health disorders (see under *Cow and calf health*), the experiment was stopped for the After-group. The BW difference between Before-calves and Control-calves was constant up to 6 weeks of age (+3.5 kg on average) and then became higher from Week 7 to Week 15 (+10.2 kg on average). From birth to weaning, Before-calves had 171 g/d higher ADG than Control-calves.

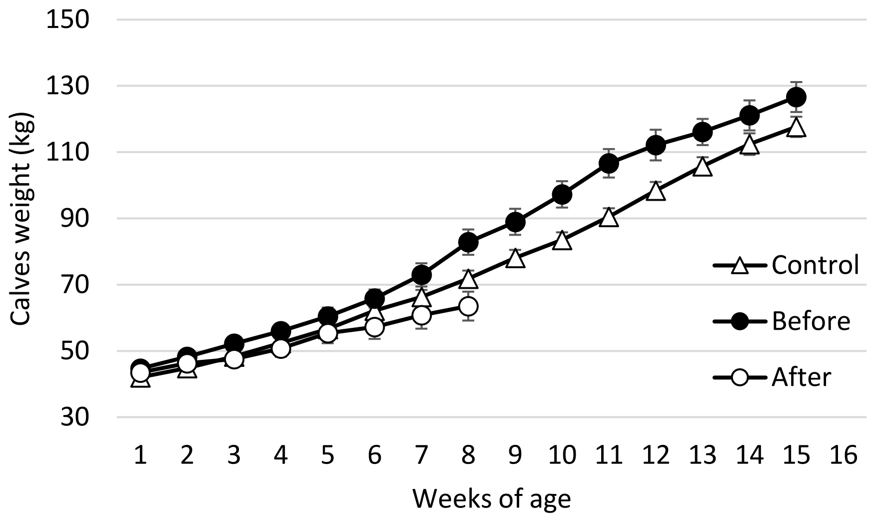

Figure 2. Weight of the calves in Trial 1 by week of age (means and standard errors of raw data). Control-calves were separated from their dam at birth, Before-calves were able to suckle their dam for 20 min before the morning milking, and After-calves were able to suckle their dam for 2.5 h after the morning milking.

Cow and calf health. During the 16 weeks of the trial, the frequency of health disorders was not significantly different between groups of cows. Reproductive disorders occurred once in Before-cows and once in After-cows, and non-reproductive disorders occurred six times in Control-cows, seven times in Before-cows and four times in After-cows. We observed statistical difference between groups of calves both in respiratory and not-respiratory disorders ($P = 0.02$ and $P = 0.001$, respectively). Three Before-calves and six After-calves were diagnosed with respiratory disorder due to bovine respiratory syncytial virus (BRSv). Despite medical treatment (anti-inflammatory drugs, facilitation of pulmonary ventilation, and antibiotics to prevent complications), two Before-calves and four After-calves died. Four After-calves had episodes of diarrhoea, including two affected by the respiratory disorder. We decided to stop the experiment for the After-calves on 12 June due to the high calf morbidity and mortality and their low weight gain.

Vocalizations at weaning. Control-calves vocalized mainly on Day 2 after weaning (75% of the calves; Figure 3). Before-calves started to vocalize earlier (92% on Day 1 *vs*. 17% of Control-calves; $P < 0.001$) and continued to vocalize later (75% *vs*. 33% on Day 4; $P = 0.041$). On Day 7, only 17% of Before-calves were still vocalizing. Before-cows started to vocalize on Day 1, but less frequently than their calves (55% *vs*. 92%; $P = 0.043$), and stopped vocalizing earlier than their calves (27% *vs*. 75% on Day 4; $P = 0.022$). On Day 7, no Before-cows were vocalizing.

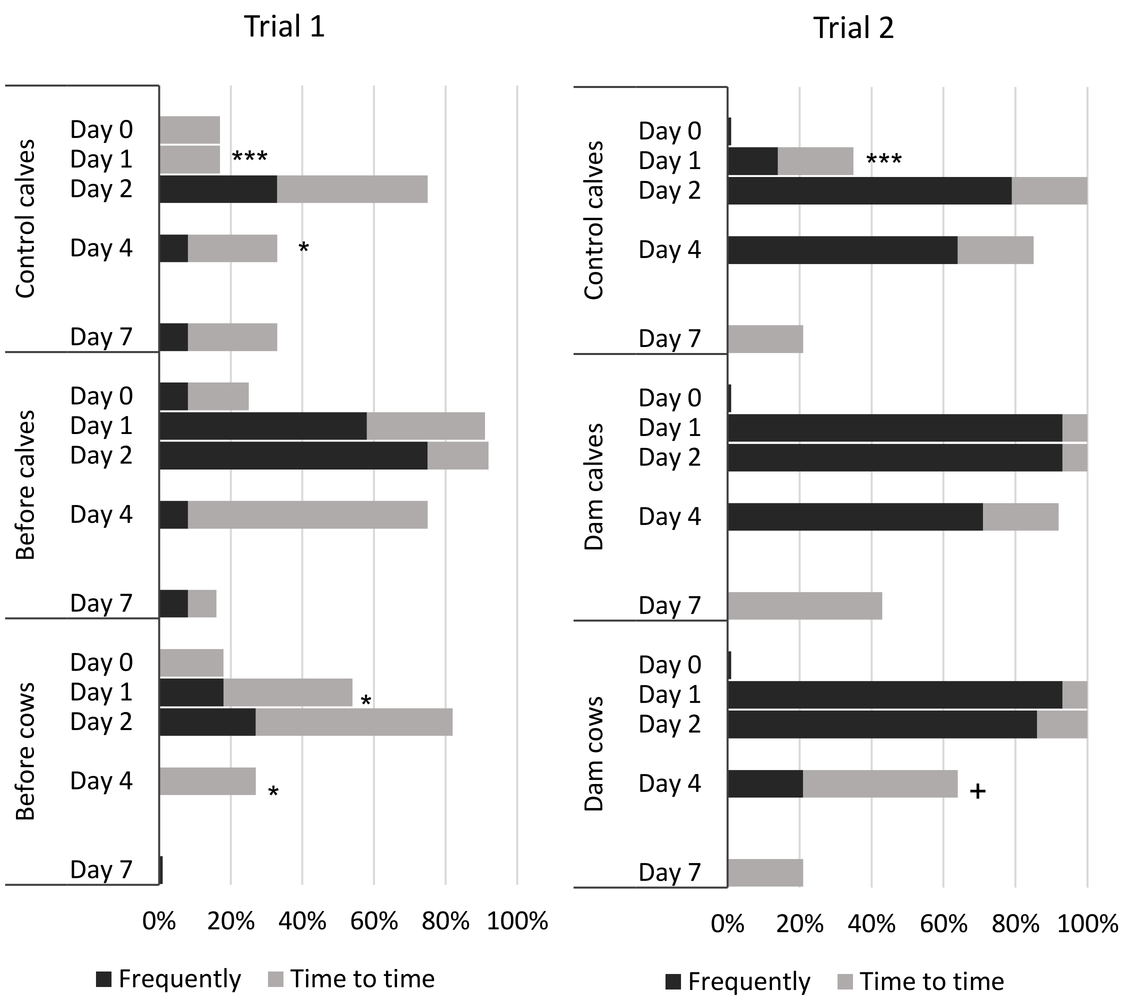

Figure 3. Percentage of animals that vocalized (*frequently* or *from time to time*) during one week around weaning (on Day 1) in Trial 1 and in Trial 2. Control-calves were separated from their dam at birth, Before-calves were able to suckle their dam for 20 min before the morning milking, After-calves were able to suckle their dam for 2.5 h after the morning milking, and Dam-calves were able to suckle their dam for 9 h between the morning and evening milkings. Chi-squared test comparing per-day data with Before-calves in Trial 1 and with Dam-calves in Trial 2.

Trial 2

Milk yield at parlour. During the first 8 weeks of lactation, Dam-cows produced 11.3 kg/d less milk than Control-cows (Table 3). The milk loss was distributed between morning (-4.1 kg/d) and evening (-7.3 kg/d) milkings. From Week 1 to Week 3, the milk yield of Dam- and Control-cows increased by 7.9 kg/d, and the difference between Control- and Dam-cows was about 9.4 kg/d (Figure 4). The milk yield of Control-cows stabilized at 28.6 kg/d from Week 3 to Week 11, and then started to decrease from Week 12 (Figure 4). The milk yield of Dam-cows decreased significantly from Week 3 to Week 8 (-3.0 kg/d; $P = 0.02$) and then increased steadily to reach the same milk yield as Control-cows on Week 14 (25.7 kg/d). The milk yield difference between Dam- and Control-cows was 10.7 kg/d from Weeks 1 to 8. During the first 8 weeks of lactation, the milk yield loss due to suckling was higher for Ho cows (-14.1 kg/d, i.e. -49%) than Mo cows (-8.4 kg/d, i.e. -34%) at both morning and evening milkings.

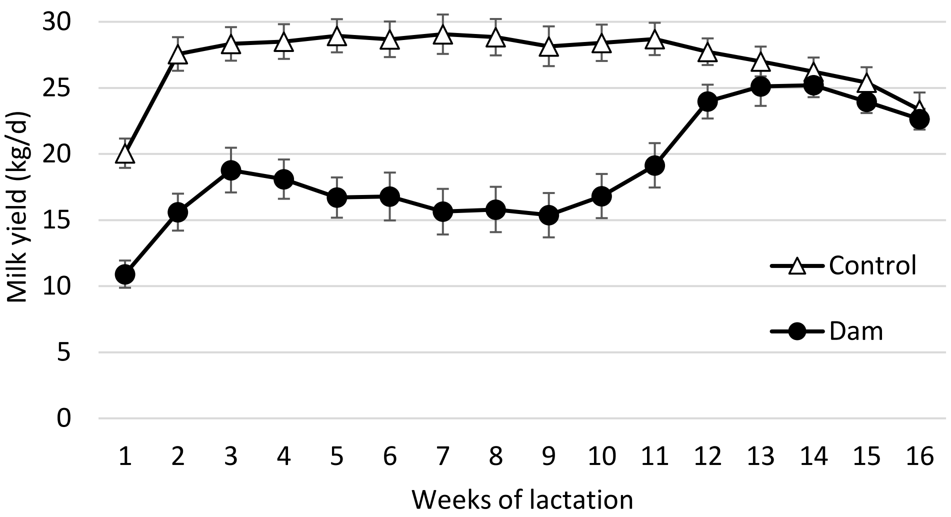

Figure 4. Daily milk yield at the parlour for the two groups of cows in Trial 2, by week of lactation (means and standard errors of raw data). In the Control-group, the calves were separated from their dam at birth and in the Dam-group, the calves were able to suckle their dam for 9 h between the morning and evening milkings. Weaning took place when the calves weighted at least 100 kg. Each group consisted of 7 Holstein (Ho) and 7 Montbéliarde (Mo) cow-calf pairs.

Milk composition at parlour. During the first 8 weeks of lactation, milk fat content was lower in Dam-cows than Control-cows (-0.92 pp), with a similar difference between morning and evening milk (Table 3). The drop in milk fat content between Dam-cows and Control-cows was more marked in Ho than in Mo, both in morning milk (-0.88 pp vs. -0.42 pp) and evening milk (-1.43 pp vs. -0.93 pp). Conversely, milk protein content was higher in Dam-cows than in Control-cows (+0.15 pp). Milk SCC was not significantly different between the two groups (38 600 cell/mL on average).

From Week 14 to Week 16, no difference were found on milk yield, milk composition and milk SCC between Dam- and Control-cows (Table 3).

Body condition of cows. At the time of calf weaning, BCS was 0.09 points higher in Dam-cows than in Control-cows, and 0.20 points higher in Mo cows than Ho cows (Table 3). The difference between Dam-cows and Control-cows was higher for Mo than for Ho (+0.16 vs. +0.02 point). At weaning, Dam- and Control-cows had similar BW (633 kg) and Ho cows weighed 9.7 kg more than Mo cows, particularly in Dam-group where Ho cows were heavier (+13.4 kg).

Calf growth. Until weaning, ADG was the same between Dam- and Control-calves and Mo calves had higher ADG than Ho calves (+289 g/d), without group × breed interaction (Table 3). Dam- and Control-calves grew similarly up to three weeks before weaning (Figure 5). During the three weeks before weaning, ADG was higher in Dam-calves than in Control-calves (+271 g/d), especially for Ho calves (+311 g/d). During the 3 weeks after weaning, ADG was lower in Dam- than Control-calves (-107 g/d). The difference was marked in Ho calves (-261 g/d; $P < 0.001$) whereas Mo Dam- and Control-calves did not differ from each other.

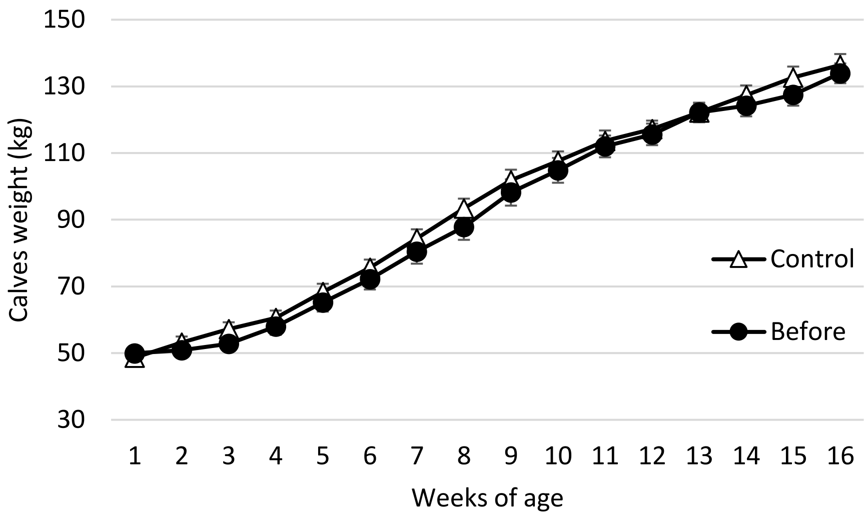

Figure 5. Weight of the calves in Trial 2 by week of age (means and standard errors of raw data). Control-calves were separated from their dam at birth and Dam-calves were able to suckle their dam for 9 h between the morning and evening milkings.

Cow and calf health. During the 16 weeks of Trial 2, the frequency of health disorders was not significantly different between Dam- and Control-cows. Reproductive disorders occurred five times in Dam-cows and two times in Control-cows, and non-reproductive disorders occurred seven times in Dam-cows and five times in Control-cows. There were no significant between-group differences in frequency of respiratory disorders (seven occurrences in Dam-calves *vs*. five in Control-calves) or non-respiratory disorders (two occurrences in Dam-calves *vs*. three in Control-calves). No calves died during Trial 2.

Vocalizations at weaning. Most of the Control-calves vocalized from Day 2 to Day 4 after weaning (100% to 86%; Figure 3). Dam-calves started vocalizing earlier than control-calves (100% of Dam-calves on Day 1 *vs*. 36% of Control-calves; $P < 0.001$) and continued vocalizing until Day 4 (93%, of which 71% vocalized frequently). On Day 7, 43% of the Dam-calves and 21% of Control-calves vocalized from time to time. All the Dam-cows started vocalizing on Day 1, but tended to stop vocalizing earlier than their calves (64% *vs*. 93% on Day 4; $P = 0.065$). On Day 7, 21% of the Dam-cows vocalized from time to time.

Acceptance of calves by cows. On average, the acceptance by Mo cows of calves other than their own was significantly higher than 1 (1.46 ± 0.14, $P < 0.05$). Conversely, the acceptance of another calf by Ho cows was not significantly higher than 1 (1.28 ± 0.14, $P > 0.05$).

Table 2. Milk production from cows and calves growth in Trial 1. Milk yield and composition of cows are given from Week 1 to Week 8 after calving - when all Before- and After-calves could suckle their dam -, and from Week 14 to 16 - when all calves where separated from their dam-. Average daily gain (ADG) of calves is calculated until 8 weeks of age and until weaning. In the Control-group, the calves were separated from their dam at birth; in the Before-group, the calves were able to suckle their dam for 20 min before the morning milking; in the After-group, the calves were able to suckle their dam for 2.5 h after the morning milking. Weaning took place when the calves weighted at least 100 kg. Each group consisted of 7 Holstein (Ho) and 7 Montbéliarde (Mo) cow-calf pairs. Adjusted values and P-values per group (Control, Before, After), per breed (Ho vs. Mo), and group × breed interactions.

	Group			Breed			*P-value*		
Week1-Week8	Control	Before	After[†]	Ho	Mo	SEM	Group	Breed	Group x Breed
Milk yield (kg/d)	24.5[c]	11.9[a]	17.4[b]	20.2	15.7	0.79	<.0001	<.0001	0.423
Morning milk yield (kg/d)	14.7[c]	3.6[a]	11.6[b]	11.2	8.75	0.45	<.0001	<.0001	0.427
Evening milk yield (kg/d)	9.9[c]	8.4[b]	5.9[a]	9.12	7.01	0.35	<.0001	<.0001	0.393
Milk fat content (%)	3.54[b]	3.86[b]	3.08[a]	3.55	3.44	0.08	<.0001	0.234	0.014
Morning milk fat content (%)	3.09[b]	2.63[a]	2.93[b]	2.94	2.83	0.08	0.0003	0.220	0.028
Evening milk fat content (%)	4.22[b]	4.25[b]	3.40[a]	4.00	3.91	0.08	<.0001	0.391	0.009
Milk protein content (%)	2.98[a]	3.28[b]	3.00[a]	2.98	3.19	0.04	<.0001	<.0001	0.597
Morning milk protein content (%)	2.98[a]	3.32[b]	3.01[a]	3.00	3.21	0.04	<.0001	<.0001	0.621
Evening milk protein content (%)	2.96[a]	3.27[b]	2.96[a]	2.97	3.15	0.05	<.0001	0.0015	0.113
Milk SCC[‡] (log_{10}/mL)	4.63	4.86	4.88	4.82	4.75	0.11	0.211	0.570	0.909
Milk ingested by calf (kg/d)	6.02[b]	7.28[c]	2.93[a]	5.39	5.43	0.24	<.0001	0.892	0.002
ADG until 8 weeks of age (g/d)	580[b]	760[c]	318[a]	491	614	41.2	<.0001	0.014	0.450
Week14-Week16									
Milk yield (kg/d)	20.6	15.4		18.4	17.6	0.88	0.0003	0.531	0.177
Milk fat content (%)	3.44	3.75		3.59	3.59	0.07	0.003	0.953	0.583
Milk protein content (%)	2.84	2.98		2.84	2.98	0.05	0.052	0.048	0.180
Milk SCC[‡] (log_{10}/mL)	4.69	4.90		5.05	4.55	0.16	0.320	0.026	0.285
ADG until weaning (g/d)	702	873		769	807	37.3	0.005	0.469	0.469

[†] Data refers only to weeks 1 to 8 after calving
[‡] Somatic cell count

Table 3. Milk production and body condition of cows and calves' growth in Trial 2. Milk yield and composition are given from Week 1 to Week 8 after calving - when all Dam-calves could suckle their dam - and from Week 14 to 16 - when all calves where separated from their dam-. Cow Body Condition Score (BCS) and Body Weight (BW) were measured at weaning. Calves were weighted once a week and their Average Daily Gain (ADG) around weaning was calculated. In the Control-group, the calves were separated from their dam at birth and in the Dam-group, the calves were able to suckle their dam for 9 h between the morning and evening milkings. Weaning took place when the calves weighted at least 100 kg. Each group consisted of 7 Holstein (Ho) and 7 Montbéliarde (Mo) cow-calf pairs. Adjusted values and P-values by group (Control, Dam), breed (Ho vs. Mo), and group × breed interactions.

	Groups		Breed			*P-value*		
Wk1-Wk8	Control	Dam	Ho	Mo	SEM	Group	Breed	Group x Breed
Milk yield (kg/d)	26.8	15.5	21.7	20.7	1.20	<.0001	0.564	0.084
Morning milk yield (kg/d)	16.2	12.1	14.4	13.9	0.94	0.004	0.671	0.137
Evening milk yield (kg/d)	10.8	3.5	7.45	6.86	0.38	<.0001	0.281	0.051
Milk fat content (%)	3.89	2.97	3.55	3.31	0.13	<.0001	0.200	0.182
Morning milk fat content (%)	3.52	2.87	3.27	3.12	0.15	0.003	0.482	0.291
Evening milk fat content (%)	4.41	3.23	4.01	3.62	0.10	<.0001	0.021	0.103
Milk protein content (%)	2.96	3.11	2.98	3.08	0.05	0.031	0.175	0.264
Morning milk protein content (%)	2.96	3.10	2.99	3.07	0.05	0.040	0.213	0.225
Evening milk protein content (%)	2.98	3.09	2.97	3.09	0.11	<.0001	0.066	0.291
Milk SCC‡ (log_{10}/mL)	4.68	4.94	4.93	4.69	0.12	0.126	0.146	0.535
Wk14-Wk16								
Milk yield (kg/d)	23.9	23.1	24.6	22.4	0.80	0.443	0.067	0.474
Milk fat content (%)	3.33	3.38	3.37	3.35	0.05	0.507	0.844	0.854
Milk protein content (%)	2.86	2.96	2.86	2.96	0.05	0.138	0.143	0.381
Milk SCC‡ (log_{10}/mL)	4.96	4.86	5.01	4.81	0.13	0.587	0.267	0.898
Cow BCS at weaning (0-5 scale)	1.50	1.59	1.44	1.64	0.02	0.045	<.0001	0.003
Cow BW at weaning (kg)	631	635	638	628	2.18	0.147	0.001	0.002
Calf ADG until weaning (g/d)	928	935	787	1076	7.6	0.533	<.0001	0.461
Calf ADG 3 weeks before weaning (g/d)	1137	1408	1083	1462	12.6	<.0001	<.0001	0.026
ADG 3 weeks after weaning (g/d)	513	406	607	312	10.8	<.0001	<.0001	<.0001

‡ Somatic cell count

Discussion

Here we tested three practices of restricted CCC that allowed suckling: two practices that allowed only short contacts during the day, i.e. just before (for 20 min/d) or after (for 2.5 h/d) the morning milking, and one practice that allowed a long period of free cow–calf contact (for 9 h/d) between the morning and evening milkings. First we discuss the pro and cons of each practice before addressing common aspects in terms of milk production and weaning.

Suckling for 2.5 h immediately after milking fails to cover the calves' nutritional needs while significantly reducing the amount of sellable milk

Allowing calves to suckle their dam for 2.5 h immediately after milking (group referred to as 'After') was tested here as a way to minimize the impact of suckling on milk yield, as we expected the calf to be able to ingest the residual milk with minimal impact on evening milk yield at the parlour. Under this practice, the cows yielded on average 29.0% less sellable milk than Controls, whereas the calves suckled relatively little milk (3.0 kg/d) and consequently had very low gain (half that of Control-calves) and poor health. When After-calves joined the cows, it is likely that not enough milk remained in the udder or that preceding machine milking prevented the occurrence of a new peak of oxytocin during suckling that would have activated let-down of the remaining milk. We had to stop this suckling practice after eight weeks to avoid putting the calves at too much risk. We concluded that there is no benefit in allowing calves to suckle for a brief duration immediately after milking.

Suckling for 20 min before milking satisfies the calves' nutritional needs but drastically reduces the amount of sellable milk

Allowing calves to suckle their dam for 20 min before milking (group referred to as 'Before') was tested here as a way to give calves a short period of access to large quantities of milk that should cover their nutritional needs. Indeed, during the first eight weeks, Before-calves ingested 20% more milk than the Control-calves that were provided milk from an automatic feeder (in quantity equivalent to 13% of their BW). Not all Control-calves ingested all the milk offered to them. Being able to suckle the dam seems thus to stimulate milk ingestion more than delivering milk by a feeder, even when the feeder is equipped with teats. Before-calves grew faster than Control-calves (ADG: +24.4% in Before-calves), which confirms earlier findings that suckling is beneficial to calf growth (e.g. Roth et al., 2009). This benefit may come from the higher amounts of milk ingested or from the positive effect of suckling on calf metabolism (Uvnäs-Moberg et al., 2001).

Milk release by cows during milking was strongly reduced when the cows suckled their calves just before the morning milking (51.4% less milk than in Control-cows). However, this decrease was still lower than that reported by Barth (2020) in cows suckling their calves just before both daily milkings (71% less milk). In our experiment, the reduction in milk yield was very marked at morning milking (75.5% less milk), which was expected because the calves started to empty the udder before milking, but was still visible at the evening milking (15.2% less milk).

Allowing cow–calf contact from morning to evening milking offers a good compromise between meeting calf nutritional requirements and preserving sellable milk

To minimize the impact of suckling on the amount of milk collected at the milking parlour while at the same time allowing calves to drink enough milk from their dam, we tested a third practice that consisted of reuniting cows and calves for 9 h a day between the morning and the evening milkings (group referred to as 'Dam'). In parallel, we increased the amount of milk offered to Control-calves (14% BW), because some of the Control-calves in Trial 1 may have not been provided enough milk as some of them drank all that was offered. On average, the Dam-cows produced 42.2% less milk than

Controls. One could expect no reduction at the morning milking because the cows and calves were separated at night, but this was not the case (reduction of 25.3%).

Dam- and Control-calves had a similar growth, which suggests they ingested similar amounts of nutrients.

Suckling reduces milk yield and affects milk composition in all scenarios

In our experiments, suckling cows always yielded less milk at the milking parlour than non-suckling cows: 29.0% less when the calves had access to the cows just after the morning milking (for 2.5 h), 42.2% less when the calves had access to the cows between milkings (for 9 h), and 51.4% less when they had access to the cows for a short period (20 min) just before the morning milking. These reduced yields are consistent with those reported in the literature: 24% less when suckling takes place for 2 h after milking (de Passillé et al., 2008), and 43% less when the calves stay with the dam between evening and morning milkings (Barth, 2020). As already reported by Fröberg et al. (2005) and Barth (2020), the reduction in milk volume collected at the milking parlour cannot be explained solely by the milk ingested by calves. Indeed, in Trial 1 where milk ingested by calves was measured, the reduction of sellable milk exceeded the amount of milk suckled by calves, and therefore total milk production was still lower than that of Control-cows, by 22% in Before-cows and 17% in After-cows. The oxytocin release at milking is less marked when cows suckle their calves, either when suckling occurs just before milking (Lupoli et al., 2001) or after milking (de Passillé et al., 2008). This could explain partly why all suckling cows in this experiment released less milk at the milking parlour even when their calves drank very little milk. Nevertheless, the decreased total milk production occurred only at the time of the lactation peak, which was absent in Before- and After-cows, whereas interestingly, total milk production of Control- and Before-cows was similar after Week 8 although calves were still suckling. This observation, never reported before, deserve to be confirmed in further trials where milk ingestion by suckled calves is measured.

In the Dam-cows, the higher reduction in milk obtained at the parlour in Ho cows compared to Mo cows could be at least partly explained by their slightly higher acceptance of calves other than their own. Moreover, the higher milk yield of Ho cows may make them more attractive to calves.

After separation from their calves, the milk yield at parlour increased in all suckling cows. Dam-cows reached the milk yield of Control-cows within a week, whereas Before-cows never managed to 'catch up' with Control-cows (-25.2% from week 14 to 16). Similar observations were reported by Barth (2020) in cows suckling their calves before milking. Cows from some breeds (like Salers or Zebu-Holstein) can only be milked if the calves first initiate milk release by a short suckling (Fröberg et al., 2007; Guiadeur et al., 2011). We suspect that this same kind of process sets in when dairy cows get used to suckle just before milking, making it more difficult to milk them at the parlour when suckling ends.

Suckling had effects on milk composition. Milk fat content decreased when cows suckled after milking or between milkings and increased when they suckled just before milking. When suckling occurs after milking, the calves mainly consume the residual milk, which has a high fat content, whereas when suckling occurs before milking, the calves suckle the cisternal milk, which has a low fat content (Fröberg et al., 2007; Tesorero et al., 2001). These variations in turn affect the fat content of the milked milk. Milk protein content increased in Before- and Dam-cows, which confirms previous results from Margerison (2002) and Barth (2020). Milk protein content increases when the energy balance of the cows is higher (Coulon & Rémond, 1991). Because suckling cows produced less total (suckled + milked) milk and presumably had similar feed intake, their energy balance was probably higher than that of Control-cows, which is consistent with the higher BCS of Dam-cows than Controls.

Suckling is thought to improve udder health (Fröberg et al., 2005; Margerison et al., 2002). However, in our studies we never found difference in SCC or frequency of mastitis between CCC practices and

Controls. As already noticed by Johnsen et al. (2016) in their review on CCC, the beneficial effect of suckling on cows udder health is not always observed.

Weaning is stressful for both calves and cows

Dam-calves showed depressed growth for 3 weeks after weaning. This could be attributed to the removal of milk from the diet while the animals are not yet used to eating significant amounts of solid feed (Weary et al., 2008). However, the drop in growth was also observed, to a lesser extent in Control-calves for which milk removal had been more progressive, allowing them to get accustomed to a solid diet. It thus appears unlikely that nutritional aspects alone fully explain the observed post-weaning reduction in growth. The stress experienced at weaning may also affect growth. Indeed, weaning has a psychological component due to the associated changes in environment: separation from the dam for calves suckling their dam, changes in accommodation (for all calves in our experiments), and changes in feeding routines (Jasper et al., 2008; Veissier et al., 1989; Weary et al., 2008). Almost all the Before-calves and Dam-calves vocalized on the day of weaning whereas Control-calves vocalized about 24 h later. Vocalizations can be at least in part due to hunger since calves vocalize less when they have access to milk after the separation from their dams (Johnsen et al., 2018). According to Thomas et al. (2001), vocalizations in the first hours after weaning are due to the separation from the dam, and vocalizations later on are due to hunger. This suggests that at weaning, Before-calves and Dam-calves react more specifically to the separation from the dam whereas Control-calves react to the lack of milk.

The cows vocalized for two days after separation, either from time to time (Before-cows) or more frequently (Dam-cows). In our study, calves were weaned according to their age and were therefore removed not all at the same time. As most cows suckled other calves after their own calves had been weaned, we can rule out the hypothesis of vocalizations reflecting discomfort produced by a distended udder due to lack of suckling after weaning. Cows establish strong bonds with their offspring, and if bonding is followed by an abrupt separation, the cows manifest stress reactions such as restlessness and vocalizations (Flower & Weary, 2001; Weary & Chua, 2000). Cow vocalizations at weaning are thus likely to reflect stress experienced by cows due to separation from their calves.

In conclusion, a short cow–calf contact (2.5 h) immediately after milking does not provide enough milk for the calves, whereas a short contact (20 min) immediately before milking strongly decreases the amount of sellable milk. Allowing a long period of CCC (9 h) between morning and evening milkings makes good compromise between sellable milk and calf growth. Contrary to what was expected, weaning induces a stress in cows and calves that have experienced restricted suckling. To promote animal welfare by allowing cow–calf contact and suckling, it is therefore necessary to reduce weaning-related stress. Restricted suckling probably needs to be followed by a two-step weaning process using nose-flaps for a few days before the separation or by using a fence-line separation to enable continued visual and some tactile contact after weaning (Haley et al., 2005; Johnsen et al., 2018; Loberg et al., 2008; Price et al., 2014).

The reduction in sellable milk and in its fat content due to suckling will affect the revenue of the farmer from the milk production. In order to assess the impact on farmers income, one should undertake a close analysis of all impacts, not only on milk production but also on calves, on cows' career, on workload, etc. If the negative impacts are not balanced by benefits then the opportunity to generate added value for this practice should be investigated, considering the demand for certification aiming at identify "animal welfare" practices or "husbandry systems" traceability (Beaver et al., 2020; Janssen et al., 2016).

Acknowledgements

The authors thank the staff of the INRAE farm at Marcenat (UE Herbipôle) for animal care, Nadège Aigueperse for her held and advice on experimental design and behavioral data processing, and Hélène

Bec for her active participation in Trial 2. This study was part of the EU project ProYoungStock funded by CORE organic. This research was supported by French government IDEX-ISITE initiative 16-IDEX-0001 (CAP 20-25). A. Nicolao received a doctoral fellowship from the Fondazione Cariparo at University of Padova (Italy).

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CHAPTER 3

Implications of cow-calf contact practices on animal health and productive performances

This Chapter presents the results of Trial 3, in which the consequences of two CCC practices on animal performance, health, and stress at weaning were investigated. It is based on a 4-pages conference paper presented at the Video Pre-Conference on Animal Husbandry at the IFOAM congress[1] on the 21-22 September 2020 (section 3.1) where animal performance results were reported. Other results obtained in Trial 3 on cows' blood metabolites, passive transfer of immunity from cows to calves, antibodies in suckling cows' milk, and hair cortisol in calves are presented in this Chapter as short papers (sections 3.2., 3.3., 3.4., respectively).

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3.1. Which compromise between milk production and cow-calf contact in dairy systems?

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Key words: dairy calves, cow-calf contact, milk production, growth of calves, behaviour

Abstract

In organic dairy farms, cow-calf contact is encouraged until weaning and requested by society. However, farmers question this practice, especially because of the loss of marketable milk. At INRAE experimental farm 'Herbipole', we tested two different suckling strategies on animal performance and behaviour. A 14-cow 'Classic' rearing system (C group) was compared during 14 weeks to two suckling systems. In the C group, calves were separated from dams immediately after birth and fed with an automatic milk feeder until weaning. In the 'Dam' group (D), dam-calf contact was allowed from birth to weaning, between morning and evening milking. In the 'Mixed' group (M), calves were kept with dams until 4 weeks (as in D group) before being separated and reared as in C group. In each group, five male calves were sold at 4 weeks of age. All calves were weaned at about 11 weeks. On average, over 14 weeks, D and M cows produced 25.1% less milk at parlour than C cows; milk fat content was 3.6 g/kg lower in D group compared to C and M, and milk protein content was intermediate between C and M. After 11 weeks, D-calves weighed 20.5 kg more than C and M calves. Cows and calves both vocalised for one week after separation or after weaning. All calf vocalisations were at a maximum during the first four days. Cows' vocalisations were less notable when calves were removed after three weeks compared to 11. In conclusion, a four-week suckling period seems better for farmers' income and cows' distress, but it induces stress for calves at both separation and weaning, without benefit on growth.

Introduction

In organic dairy farms, cow-calf contact is encouraged until weaning and requested by society (Agenäs et al. 2017). However, calves are usually fed natural milk until 12 weeks, instead of maternal milk as suggested by the organic guidelines, due to being separated from their dam shortly after birth. Long-term cow-calf contact can promote animal welfare and improve health and growth of calves (Roth et al. 2009). By contrast, a short contact period decreases the marketable milk loss for the farmer and is believed to reduce stress at separation. The aim of this study was to investigate a rearing system that would represent the best compromise between cow-calf contact, animals' performance and stress at separation or weaning.

Material and methods

The experiment took place in 2019 at the INRAE Herbipôle experimental farm (DOI: https://doi.org/10.15454/1.5572318050509348E12), located in Marcenat (F-15114,

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45.30°N, 2.84°E, 1080 m a.s.l.). All procedures were approved by the local animal ethic committee and followed the guidelines for animal research of the French Ministry of Agriculture and all other applicable national and European regulations.

During 14 weeks after calving, performance and behaviour of three groups of 14 cows and corresponding calves were compared. In each group, five male calves were sold at 4 weeks of age. Cows groups were balanced according to breed (Holstein or Montbéliarde), lactation rank, date of calving, and milk index. The 42 cows were housed in the same free-stall barn, divided into three pens. They calved regularly from 12 February to 5 May and moved to the milking parlour each day at 6:30 and 15:30. They were fed *ad libitum* with a mixed ration (59% 1st-cut hay; 32% 2nd-cut hay; 9% concentrate), plus 2 kg/d of concentrate per cow. From the 5 May, cows were grazing day and night and received 2 kg/d of concentrate. In the Control group (**C**), calves were separated at birth and received at least two litres of fresh colostrum with a feeding bottle (or thawed and reheated if the quality was <24% Brix, measured by refractometer). Calves were housed in individual pens for seven days and bucket-fed bulk milk twice a day. They were thereafter placed in a collective straw-bedded pen with a hay rack and received bulk milk and concentrate by automatic feeders according to a feeding plan *(milk: 6 to 9 kg/d from wk1 to wk3; 10 kg/d from wk4 to wk6; 9 to 3 kg/d from wk7 to wk10 – concentrate: 0.2 to 2.0 kg/d from wk3 to wk10)*. In the 'Dam' group (**D**), calves spent five days after birth in individual calving pens with their dam and received colostrum directly from their dam. Thereafter, D-calves were housed in a collective straw-bedded pen next to their dam. From 9:00 to 15:30, calves had free access to the D-cowshed where they were suckled by the dams. From 15:30 to 9:00, the separation gate was closed but calves could see their dam. Calves had free access to a hay rack and a bucket of concentrate. In the 'Mixed' group (**M**), calves were reared as calves of the D group until 4 weeks of age, before being separated and reared as calves of the C group. The day of separation, calves were moved to a remote pen, and cows stayed in the collective barn with the remaining dam-calf pairs.

Five male calves of each group were removed from their respective pen after 21 days of age, to be sold. Remaining calves were weaned when they weighed about 100 kg (corresponding to 11 weeks of age, on average) and were moved to collective pens. Separation of males for selling, separation of M-calves from their dam and weaning took place in waves every two weeks during the Tuesday morning milking.

Individual milk yield was measured twice a day at the milking parlour with DeLaval flow meters. Milk fat and protein contents were determined weekly on four consecutive milkings by mid-infrared spectroscopy and milk somatic cell count (**SCC**) was measured by epifluorescence on two consecutive milkings. This allowed for calculation of the average individual milk yield and composition by week of lactation. Calves were weighed at birth and then weekly until 14 weeks. We observed the behaviour of calves and cows at separation (M group) and at weaning (except C-cows), during one week: the day before the separation/weaning (Day0), the day of the separation/weaning (Day1), and on days 2, 4 and 7. Calves and their respective dam were observed twice a day at the same time, in the morning and in the evening, during two periods of 5 min to note if they vocalised or not.

The data were analysed using the MIXED procedure of SAS software. The model for milk included the effects of the cow (random factor), rearing group (Classic, Dam or Mixed), breed (Holstein or Montbeliarde), parity (primiparous or multiparous), week of lactation (repeated factor), date of calving, and the group*breed and group*week interactions. For the analysis of calves' weight around weaning (week 11, on average), the model included the effects of rearing group, breed, calf sex, date of birth, birth weight and the group*breed interaction. Finally, we calculated the daily percentage of animals that vocalised, by group and by type of animal (calf or cow).

Results

Milk yield at parlour of M-cows was similar to that of D-cows until the separation of calves (week 4). From there, production increased to reach that of C-cows on week 9 (Figure 1). Milk yield at parlour of D-cows increased after the weaning of the last calves (week 12) to reach that of M- and C-cows. Over 14 weeks, M- and D-cows produced 20.5% and 29.7% less milk at parlour than C-cows (Table 1). Milk fat content was lower in D group compared to C and M groups (-3.6 g/kg, on average) and protein content was lower in C group compared to D group (-1.0 g/kg; P=0.089) and M group (-1.6 g/kg; P=0.011). Milk SCC was not significantly different between the three groups.
Until four weeks of age, the growth of the three groups of calves was identical (616 g/d; Figure 2). Between weeks 5 to 11, D-calves had a higher growth (1139 g/d) than M and C-calves (845 and 875 g/d). At 11 weeks of age, around weaning, D-calves weighed on average 20.5 kg more than M- and C-calves (120.3 vs 99.5 kg, on average ; P<0.001). After weaning, the growth of D-calves dramatically slowed down (266 g/d between weeks 11 and 14) whereas C- and M-calves were less impacted (641 g/d on average). On week 14, D-calves still weighed 8.9 kg more than M- and C-calves (P<0.05).

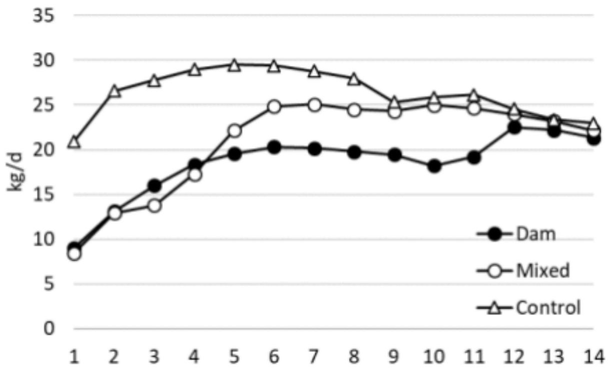

Figure 1. Average daily milk yield at parlour by week of lactation

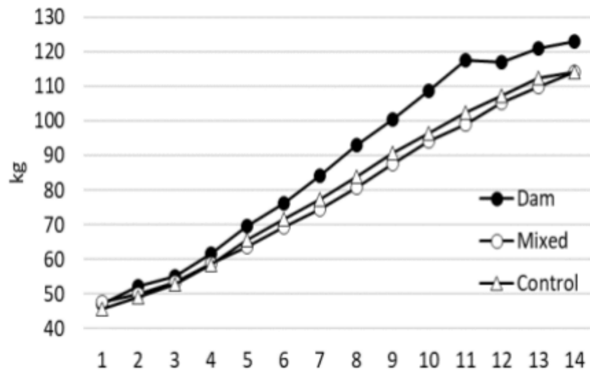

Figure 2. Average weight of calves by week of age

Table 1: Average milk yield and composition during the first 14 weeks after calving, according to the rearing group *(adjusted values)*

	Control	Mixed	Dam	P-value
Milk yield at parlour (kg/d)	26.3[a]	20.9[b]	18.5[b]	<0.001
Milk fat content (g/kg)	37.2[a]	36.3[a]	33.2[b]	0.007
Milk protein content (g/kg)	29.9[a]	31.5[b]	30.9[ab]	0.034
Milk Somatic cell count ($\log_{10}$/mL)	4.73	4.82	4.84	0.817

[a-b] Means within a row with different superscript letters differ at P<0.05

After separation, in M and D groups, the percentage of cows and calves that vocalised was the highest on days 1 and 2 (54% and 91% on average for cows and calves, respectively), then it decreased regularly until Day7 (Figure 3). From Day1 to Day7 it was higher for D-cows (6.7-point) and calves (14.7-point) compared to M-cows and calves. After weaning, the percentage of calves that vocalised was the highest on Day2 (94%, on average), then it decreased regularly until Day7 (Figure 4). On Day1, this percentage was two times higher for D-calves (93%) than for C- and M-calves (46%, on average). Conversely, on Day7, the percentage of calves that vocalised was more than two times higher in C group (42%) than in D and M groups (15%).

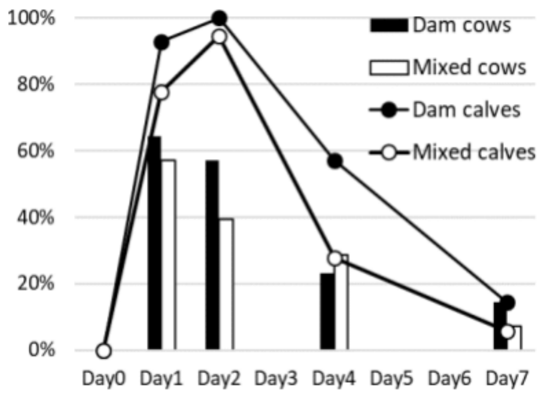

Figure 3. Percentage of animals that vocalised after separation (Day1)

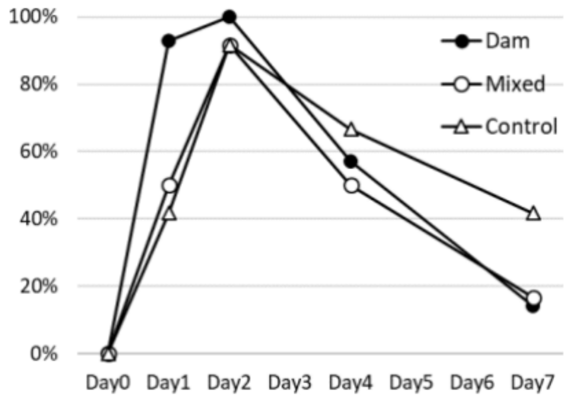

Figure 4. Percentage of calves that vocalised after weaning (Day1)

Discussion

Compared to a classic rearing system of dairy calves, both suckling systems affected production: less milk was collected at the parlour, with a lower fat content in the case of very late separation, and a higher protein content. This loss is higher (764 kg by cow; adjusted value) than the amount of milk consumed by the same number of calves fed bulk milk with an automatic feeder (396 kg by cow; raw data). For a farmer, this difference represents only about 6.1% of the total production of his herd (considering 6 000 kg by lactation). However, a long-term contact with dams improved the growth of calves, which was not the case with a short contact period. This may be due to the stress of the separation combined with the need to cope with the automatic feeder. Separation and weaning are very stressful events for calves because, whatever the group, they all vocalised the following day. Separation seemed less stressful for cows, especially when calves were removed after a few weeks. However, whether at separation or at weaning, some cows and calves still vocalised one week after. In conclusion, a four-week suckling period seems better for farmers' income and cows' distress, but it induces two periods of stress for calves (separation then weaning) instead of one (simultaneous separation and weaning), without benefit on growth. Conversely, the coincidence of separation and weaning after three months induces a strong slowdown of calves' growth.

Suggestions for research and support policies to develop further organic animal husbandry

Rearing dairy calves with their dam until weaning, or at least for few weeks, must be more strongly encouraged in organic farming. For the farmer, marketable milk loss should be compensated by a higher price for a milk labelled "from cows suckling their calves". Further research on the implementation of a gradual weaning, or a weaning without separation, is needed to reduce the stress of both calves and cows. [1]

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3.2. Blood metabolites

3.2.1. Introduction

The ability of ruminants to cope with periods of food scarcity is the result of a long evolutionary process under harsh natural conditions, and further modified by human intervention through the selection of specialized breeds (Chilliard et al., 2000). When animals are underfed, they mobilize their body reserves, which then need to be restored during the more favourable season to allow them to enter in the next cycle of reproduction. This mobilization of body reserves, which occurs even in non-productive animals, is amplified during pregnancy and lactation by increased physiological needs (Chilliard et al., 2000). The early lactation is characterized by a rapid increase in milk production, mobilization of body protein and fat reserves, a negative energy balance (NEB), and changes in the protein, fat, and fatty acid (FA) composition of milk (Billa et al., 2020). In this period, the occurrence of metabolic disorders related to energy metabolism is usual, and the classic indicators for herd troubleshooting are plasma concentrations of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) (Oetzel, 2004). In addition, several factors can compound this natural cycle of body reserves by increasing the energy deficit, which increases the mobilization and lengthens the period of negative energy balance (Larsen et al., 2016). Such factors are, for example, diseases that reduce appetite and consequently energy intake, such as ketosis or systemic mastitis (Bareille et al., 2003), or displaced abomasum (LeBlanc et al., 2005). Blood variables (glucose, NEFA, BHB, etc.) that reflect the rate and extent of tissue mobilization can be used to predict the energy status of the animal (Larsen et al., 2016).

Also suckling is recognised as one of the main factors affecting the postpartum period of beef cattle (Quintans et al., 2010) while there is a lack of studies on energy mobilization of dairy suckling cows. Bar-Peled et al. (1998) investigated the effects of frequent udder emptying, either by milking or suckling, on the DMI and digestibility of the diet. They concluded that cows suckled three times and milked three times a day had a severe negative energy balance, expressed by a heavy loss of body weight (BW), elevated NEFA concentrations, and decreased glucose concentrations in blood compared to cows milked three or six times a day.

In Trial 3, additional measurements on cow blood metabolites (not reported into the previous publication), were made in order to try to answer the question: “does the energy balance of the suckled cows explain their higher milk protein content?”. Our hypothesis was that, because suckling cows produced less total milk (suckled + milked) (see Chapter 2) and presumably had similar feed intake, their energy balance was probably higher than that of Control-cows. This could explain the higher milk protein content of suckling cows, as energy balance is one of the main drivers of milk protein content (Coulon & Rémond, 1991). In addition, this hypothesis was supported by the higher BCS of Dam-cows found in Trials 2 and 3 but this hypothesis has never been tested.

3.2.2. Material and Methods

Individual blood samples were taken from the tail vein of the cows after the morning milking with EDTA containing tubes (Terumo France, Guyancourt, France) at weeks -1 (before calving), 3 (before separation), 10 (before weaning) and 13 (after weaning) after calving. Samples were centrifuged at 1.200 × g for 20 min at 4°C and the obtained plasma immediately stored at –20°C. After thawing, the plasma concentrations of non-esterified fatty acids (NEFA; Kit NEFA-HR2, Fujifilm WAKO), glucose (Kit 981379, ThermoScientific), urea (Kit 981818, ThermoScientific) and BHB (Kit 984325, ThermoScientific) were analyzed on a chemistry analyzer (Arena 20 XT Chemistry System, ThermoScientific, Walthan).

Statistical analyses. The data were analyzed using the MIXED procedure of the SAS 9.4 software package (SAS Institute Inc., Cary, NC). Cows were considered as statistical unit and used in the models as random factors. The model took into account the effects of Group (Control group and two suckling groups), Breed (Holstein [Ho] or Montbéliarde [Mo]) and interactions Group × Week of lactation as fixed factors, week of lactation (3, 10, 13) as repeated factor and results obtained one week before calving as covariate. For all data, normality of residuals was checked using the Shapiro-Wilk test.

3.2.3. Results and discussion

Significant Week effects were observed for plasma NEFA, BHB and Glucose concentrations while Group had no significant effect on plasma metabolites (Table 3.1). Plasma NEFA and BHB significantly decreased from Week 3 to Week 10 and 13 (Figure 3.1) while plasma glucose decreased from Week 3 to Week 10 and increased from Week 10 to Week 13 (result not shown).

The higher plasma concentrations of NEFA and BHB observed in Week 3 were expected because cows were at lactation peak, when the energy balance is the most negative. Indeed, the higher plasma NEFA concentration observed in Week 3 reflects directly lipomobilisation (Chilliard et al., 2000) that remained relatively low in comparison to other studies where severe undernutrition was applied (Billa et al., 2020). As plasma BHB originates in part from rumen butyrate, (Miettinen and Huhtanen, 1996), its higher concentration observed in Week 3 probably reflects concomitant modifications of intake, possibly lower in Week 3, and ruminal butyrate synthesis as well as incomplete β-oxidation of mobilized NEFA. The plasma BHB concentrations remained nevertheless below the 1.2 mmol threshold of subclinical ketosis (LeBlanc et al., 2005) except for one cow from the Control group in Week 3 (2.16 mmol).

Table 3.1. Blood plasma metabolites of the three groups of cows. Adjusted values and P-values per Group (Control, Mixed, Dam), per Breed (Ho and Mo) and Week of lactation (3, 10, 13).

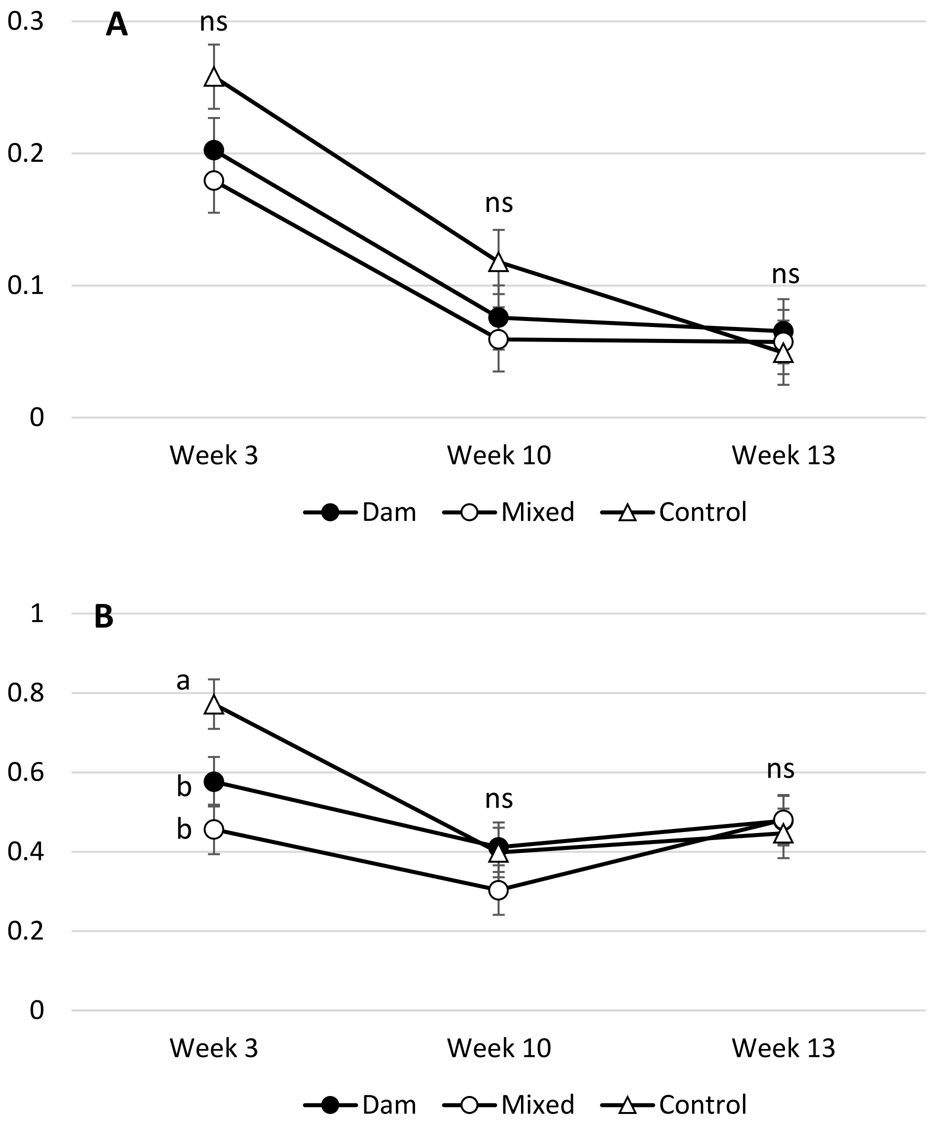
	Group				*P-Value*		
	Dam	Mixed	Control	SEM	Group	Breed	Week
Blood plasma metabolites							
NEFA[1] (mmol/L)	0.11	0.10	0.14	0.015	0.16	0.07	<0.001
BHB[2] (mmol/L)	0.49	0.51	0.54	0.058	0.10	0.60	<0.001
Glucose (g/L)	0.45	0.42	0.46	0.016	0.14	0.05	<0.001
Urea (g/L)	0.19	0.20	0.23	0.011	0.11	0.03	0.27

[1] Non-esterified fatty acids

[2] Beta-hydroxybutyrate

The cow calf contact (CCC) practices tested in Dam and Mixed groups did not affect significantly plasma metabolites but plasma BHB concentration was higher in Week 3 in the Control group in comparison to Dam and Mixed groups (significant interaction, Figure 3.1B). As animal feeding was similar in the 3 groups of cows, this higher plasma BHB concentration could reflect an incomplete β-oxidation of higher amount of NEFA mobilized by Control cows. However, our hypothesis of a lower energetic balance of Control cows is not validated by plasma NEFA that were not significantly higher in Control cows during Week 3 despite the numeric differences observed (Figure 3.1A).

Figure 3.1. Evolution from Week 3 to Week 13 of plasma concentration in NEFA (Figure A) and in BHB (Figure B) in cows according to Group (Dam, Mixed, Control). Results are expressed in mmol/L.



In conclusion, the analyses of the blood plasma metabolites made in this study do not confirm our hypothesis that CCC cows have a higher energetic balance at lactation peak that could explain their higher milk protein content. The latter could be the consequence of a dilution effect as the total milk production of CCC cows is lower from Week 1 to Week 8 (see results from Trial 1 where total milk production was estimated thanks to the measurement of milk drunk by calves).

3.3. Passive transfer of immunity from cows to calves and antibodies in suckling cows' milk

3.3.1. Introduction

A common belief is that separating the calf at birth limits the risk of transmission of diseases and that is better for both calf and cow health. The efficiency of calf cow contact (CCC) systems in achieving passive immune transfer is partly controversially discussed: on one hand, sucking of calves by their dam is thought to be less efficient in controlling colostrum ingestion (Beaver et al., 2019). On the other hand, some studies report that calves left with the dam have higher levels of immunoglobulin G (IgG) absorption and serum IgG concentrations than calves bottle-fed colostrum (Selman et al., 1971; Stott et al., 1979), while others did not identify differences in IgG concentration in rearing systems with longer cow contact (Hillmann et al. 2019). The calf's immunologic resistance to diseases during the first few weeks of life depends mainly on the amount of passive maternal immunoglobulin acquired by the calf (Besser, 1998). Consequently, colostrum-derived passive immunity is vital to the health, performance, and welfare of neonatal calves (Mcgee & Earley, 2019). Calves are born agammaglobulinemic because the cow's placenta prevents IgG transmission in utero (Godden, 2008). Consequently, the transfer of sufficient IgG to the neonatal calf through colostrum is essential to provide the calf with immunological protection and resistance against disease (Conneely et al., 2013). IgA is generally considered the primary immunoglobulin in the gastrointestinal tract (Besser, 1998). However, the absolute concentration of IgA and IgG in the intestine are similar, and IgG is the primary immunoglobulin in the intestine of the newborn calf (Besser, 1998). Transfer of IgG, which represents 85% to 95% of total immunoglobulins (Larson et al., 1980), occurs from the blood through the mammary epithelium and accumulates in the mammary gland before parturition (Conneely et al., 2013). Bovine colostrum and milk also contain much smaller amounts of locally produced IgA and IgM (Larson et al., 1980). IgG levels of < 10 g/L at 24–48 h of age in calves' serum indicate an inadequate transfer of IgG (Besser et al., 1991). An optimum colostrum management is required within the few hours after birth to allow calves receiving a sufficient amount of clean and high-quality colostrum (Johnsen et al., 2019). Some studies report that calves left with the dam have higher levels of IgG absorption and serum IgG concentrations than calves bottle-fed colostrum (Selman et al., 1971; Stott et al., 1979). In any case, the main factors to obtain an optimal transfer of immunity are the timing of colostrum ingestion, the quantity and the quality of colostrum and, when possible, the presence of the dam (Johnsen et al., 2019). When calves are allowed to suckle colostrum directly from the dam, factors such as rapidity to stand, to walk and to find teats or good maternal bond, participate in the success of rapid colostrum ingestion (Mcgee & Earley, 2019). After cessation of macromolecular transport of immunoglobulins, colostral immunoglobulins exert a local protective action in the intestine (Corley et al., 1977), as transmigration of pathogenic bacteria can be prevented by colostrum in the intestinal lumen (Bush & Staley, 1980). This form of protection can be effected in calves that are no longer able to absorb intact immunoglobulins. There is strong evidence that IgG initially absorbed by colostrum in the bloodstream is re-secreted in the intestine by crypt cells. These IgG help to reduce the incidence of different types of gastrointestinal infections, including enteropathogenic Escherichia coli, rotavirus and Cryptosporidium parvum (Besser, 1998; Quigley, 2004).

Suckling is also thought to improve udder health (Fröberg et al., 2005; Margerison et al., 2002). Several studies reported that suckling decreases the milk somatic cell counts (SCC) during the suckling period and this can be related to a lower mastitis incidence (Boden & Leaver, 1994; Krohn, 2001; Margerison et al., 2002).

In addition to SCC, another factor in determining udder health could be lactoferrin (LF). LF is a bioactive multifunctional protein of the transferrin family. LF is present mainly in the secretions of all mammals, especially in milk (Wang et al., 2021). In milk of lactating animals, LF plays a key role in mammary gland defense mechanisms (Cheng et al., 2008). LF participates in bacterial infections because bacteria require iron for growth, and under certain conditions, LF can inhibit bacteria by chelating iron (Weinberg, 1978). Some studies found a correlation between SCC and LF (Cheng et al., 2008; Harmon et al., 1975). This finding suggests that milk LF may be helpful as an indicator for intramammary infection in dairy cows (Cheng et al., 2008). For neonatal calves, milk LF, thanks to its broad spectrum of anti-microbial properties and to its regulatory functions in the immune system may also play a role in calf health prevention. It was demonstrated that calves receiving LF supplemented milk had less days of disease with less serious cases of diarrhea as well as higher weight gains (Prenner et al., 2007). In humans, the role of breast milk LF in the prevention of gastrointestinal and respiratory pathogens in young children is well documented (Manzoni et al., 2018). We hypothesized that similar mechanisms could exist also in bovine.

Finally, suckling seems to improve the health of animals, or at least this is the perception of farmers and one of the main motivations that leads them to implement this practice (Michaud et al., 2018).

3.3.2. Material and Method

We investigated the IgG concentration in serum of calves to find out if there were differences in passive immunity transfer in calves ingesting colostrum directly from their dams (Dam- and Mixed- calves: quality of colostrum was not controlled) and calves that ingest colostrum from a feeding bottle (Control-calves: good quality colostrum - controlled quality). Control-calves received at least 2.0 L of fresh colostrum from a feeding bottle. If there was no good-quality fresh colostrum available (< 24% Brix, measured by refractometer), then good-quality thawed and reheated colostrum was provided. Dam- and Mixed calves received colostrum directly from their dams and animal caretakers controlled at least twice a day if the calves suckled properly colostrum from their dam.

Calf serum was also sampled at Week 3 (before separation for mixed calves) in all the calves, and at Week 10 (before weaning) only in female calves. Similarly, every two weeks (Weeks 3, 7, 9 and 13) we sampled milk from all the cows in order to investigate the concentration of IgG and lactoferrin in milk during the suckling period. The aim was to study whether milk concentration in antibodies and antimicrobials varies between suckling and non-suckling cows.
Finally, animal caretakers checked clinical signs on animals at least once a day. They applied Standard Operating Procedures to cure the animal affected and recorded the disorder and the treatment used in a sanitary logbook. Health disorders were sorted into reproductive disorders (metritis, retention of the placental membrane, ovarian cysts, and vaginitis) and non-reproductive disorders (mastitis, milk fever, lameness, etc.) for cows, and respiratory disorders (runny nose, coughing, dyspnoea, etc.) and non-respiratory disorders (diarrhoea, umbilical infection, etc.) for calves.

Colostrum. Colostrum samples were taken in 30 mL dry vials/tubes at the first milking after calving and at the evening milking three days after calving. Samples were frozen at -20°C until the analysis of immunoglobulin G (IgG).

Milk for IgG analyses. Milk samples were taken in 30 mL non-bronopolized vials/tubes every two weeks (fixed date) during the 91 days of the trial twice, a day (one sample at the morning milking and one sample at the evening milking) with the aim of collecting at least three samples for each cow at Weeks 3, 7, 9 and 13. Samples were frozen at -20°C until the analysis of immunoglobulin G (IgG).

Milk Lactoferrin analyses. Milk samples were taken in 30 mL non-bronopolized vials/tubes at Week 3 during the morning milking the day before and the day after the separation of the calves, then once at

Week 6 during the morning milking and once at Week 13 after the weaning of all the calves. Samples were frozen at -20°C until the analysis of lactoferrin.

Serum of calves. Individual blood samples were taken at the jugular vein into 10 mL vacutainer tubes. Sampling occurred in all the calves at 48 hours after birth, then at Week 3, just before the separation of male calves in group Dam and all calves in group Mixed and finally, just before weaning (Week 10) only in female calves. Blood samples were immediately centrifuged for 20 min at 3000 × g and 4°C. Serum was pipetted into approximately 1 mL into 3 x 1.5 mL cups for each tube (= 9 cups) and frozen at −20°C for later IgG analysis.

All milk samples were analysed in the Agrolabs' laboratory (Aurillac, France). An enzyme-linked immunosorbent assay (ELISA) was used to determine lactoferrin content (mg/L) and a radial immunodiffusion method (Bovine IgG1 Test from IDBiotech, Issoire, France) to determine immunglobulin G content (mg/L) in cow milk. All blood serum samples were analysed in the VetAgro Sup laboratory (Marcy l'Etoile, France), applying a radial immunodiffusion method (Bovine IgG1 Test from IDBiotech, Issoire, France) to determine the immunoglobulin G content (IgG, in mg/dL).

Statistical analyses. All data were analysed using the SAS 9.4 software package (SAS Institute Inc., Cary, NC). The colostrum samples collected on day 1 and 3 were analysed separately using the GLM procedure with animal group (Control, Dam and Mixt) and breed (Holstein and Montbéliarde) included in the model. Data related to milk were analysed using the MIXED procedure, with animal as random factor, group (Control, Dam and Mixt), breed (Holstein and Montbéliarde), milking (morning, evening) and interaction Week x Group as fixed factors and date (Week 3, 7, 9 and 13) as repeated factor. Data related to blood plasma were analysed using the MIXED procedure, with animal as random factor, group (Control, Dam and Mixt), breed (Holstein and Montbéliarde), sex (male, female) and interaction Week x Group as fixed factors and date (day 2, Week 3 and 10) as repeated factor. For all data, normality of residuals was checked using the Shapiro-Wilk test. Frequency of health disorders around weaning was compared between groups using a Chi-squared test. Significance was set at $P < 0.05$.

3.3.3. Results and discussion

Colostrum. At Day1, Control-cows tended to have a higher concentration of IgG in colostrum at first milking after calving (Table 3.2). No Group effect was found for IgG concentration of colostrum at Day3, and no Breed effects were observed at Day1 and Day3 after calving. Suckling cows (Dam- and Mixed-) had lower IgG concentration of colostrum at Day1 probably because the IgG-richest colostrum was already suckled by calves before the first milking while it was not the case of Control-cows. The IgG concentration in colostrum is known indeed to very rapidly decrease (Johnsen et al., 2019) during the first days post-partum, as we observed in this experiment (Table 3.2).

Table 3.2. IgG concentration (g/L) of colostrum of the three groups of cows at the first milking after calving (Day1) and three days after calving (Day3). Adjusted values and P-values per Group (Control, Mixed, Dam), per Breed (Ho and Mo).

	Group				Breed		*P-Value*	
	Control	Mixed	Dam	SEM	Ho	Mo	Group	Breed
IgG colostrum d1 (g/L)	61.0	39.2	44.6	6.46	51.3	45.2	0.07	0.43
IgG colostrum d3 (g/L)	1.61	1.53	1.71	0.19	1.67	1.57	0.81	0.69

Serum of calves. The IgG concentration in calves' serum (Table 3.3) was similar in the different groups and no interaction was observed with the Week. At Day 2, in all groups, the average IgG levels were high (Figure 3.2), which indicates that the passive immunity transfer was adequate for most calves, except for 3, 2 and 4 calves (out of 14) in groups Control, Dam and Mixed respectively whose plasma IgG concentration was below the threshold of 10 g/L (Besser et al., 1991). The plasma IgG concentration decreased significantly from Week 3 to Week 10 (Figure 3.2) and was significantly higher in female than in male calves (18.2 ± 1.2 *vs* 14.1 ± 1.3 g/L, P= 0.03).

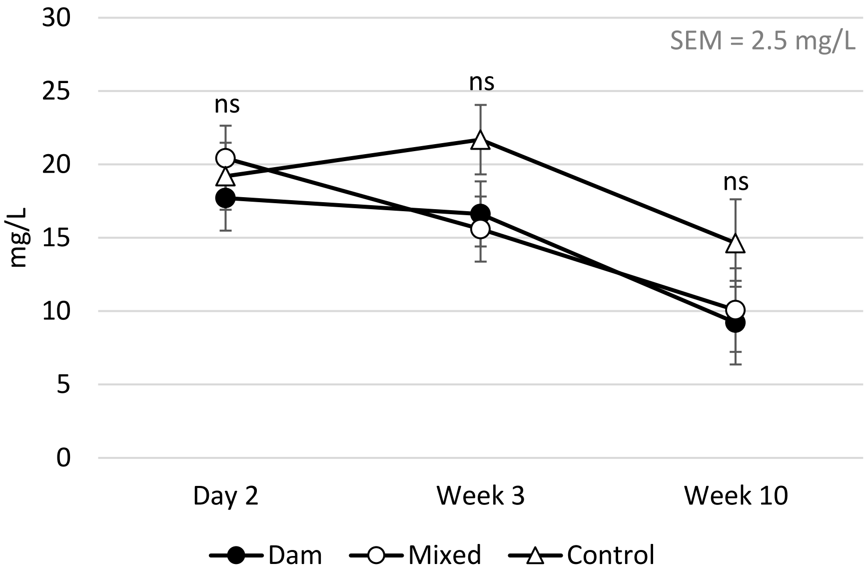


Figure 3.2. IgG serum of calves concentration (average and standard error) of the three groups of calves at Day 2 after birth, at Week 3 (separation of Mixed-calves) and at Week 10 (weaning).

Table 3.3. IgG serum of calves concentration (mg/L) of the three groups of calves (Control, Mixed, Dam). Adjusted values and P-values of IgG serum per Group (Control, Mixed, Dam), per Breed (Ho and Mo), Sex of calves and Week of age.

	Group				*P-Value*			
	Control	Mixed	Dam	SEM	Group	Breed	Sex	Week
IgG Serum (mg/L)	18.5	14.5	15.4	1.5	0.18	0.37	0.03	0.0002

We conclude from these measurements that in our experimental conditions, the passive immunity transfer from cows to calves was adequate in all groups and that Dam and Mixed CCC practices implemented in this experiment had no adverse effect on neonatal calves' immunity and no effect on the further build-up of the active immune defense during the pre-weaning period.

Immunoglobulin in cows' milk. Milk IgG concentration was significantly higher in Mixed-cows in Week 3, in comparison to Control and Dam-cows (Table 3.4 and Figure 3.3). Milk IgG concentration clearly decreased after Week 3 where group-differences observed in Week 3 disappeared. The time related decrease observed in this experiment is in line with Auldist et al. (1998) who observed that subsequently to the colostrum phase, immunoglobulin concentrations in milk decrease significantly, but increase again during late lactation. Conversely, the higher IgG concentration found only in Mixed cows is very surprising considering that in Week 3, all Mixed and Dam cows were suckling their calves. Therefore, differences do not seem to be due to calves' suckling. Further investigations need to be done in order to try to understand this surprising result that could be linked to different SCC content of milks, as previously demonstrated by Levieux (1999). Therefore, cows' suckling does not seem to have any influence on the IgG concentration of milk.

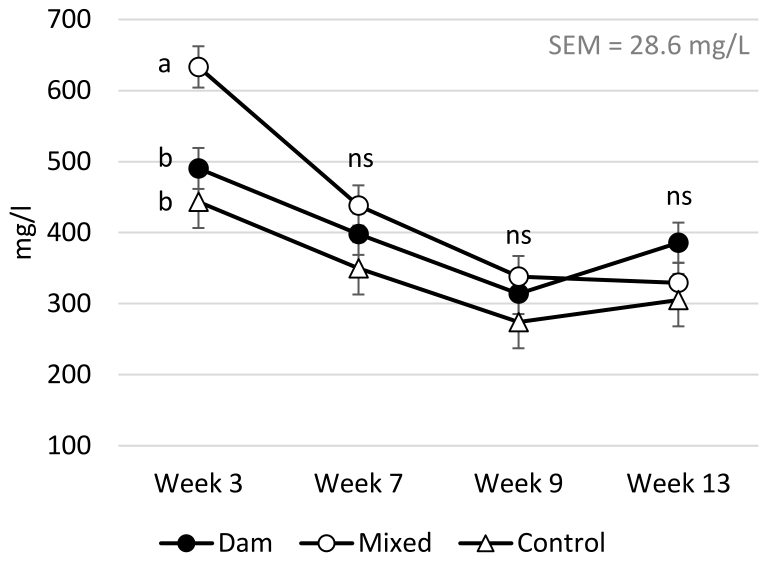


Figure 3.3. Milk IgG concentration (average and standard error) of the three groups of cows by Weeks of lactation.

Table 3.4. Milk IgG concentration (mg/L) of the three groups of calves (Control, Mixed, Dam). Adjusted values and P-values of IgG milk concentration per Group (Control, Mixed, Dam), per Breed (Ho and Mo) and Week of lactation (3, 7, 9 and 13).

	Group				*P-Value*		
	Control	Mixed	Dam	SEM	Group	Breed	Week
IgG Milk (mg/L)	342.9	433.3	395.4	23.8	0.03	0.93	<0.001

Lactoferrin in cows' milk. Milk LF concentration increased steadily from Week 3 to Week 13 and in Week 13, milk LF was significantly higher in Control-cows compared to Dam and Mixed-cows (Table 3.4, Figure 3.5). The increase of LF concentration in milk during lactation was previously reported (Cheng et al., 2008) but the higher milk LF content in Control cows in Week 13 is very surprising. It does not seem to be related to calves' suckling as in Week 13, all calves were weaned in all groups. Here again, further investigation need to be done in order to try to understand this surprising result that could be linked to milk SCC (Cheng et al., 2008), slightly higher in average in Control cows in Week 13.

Table 3.5. Milk lactoferrin concentration (mg/L) of the three groups of calves. Adjusted values and P-values per Group (Control, Mixed, Dam), per Breed (Ho and Mo) and Week of lactation (3, 7, 9 and 13).

	Group				*P-Value*		
	Control	Mixed	Dam	SEM	Group	Breed	Week
Milk Lactoferrin (mg/L)	195.7	137.7	149.3	21.6	0.13	0.13	<0.001

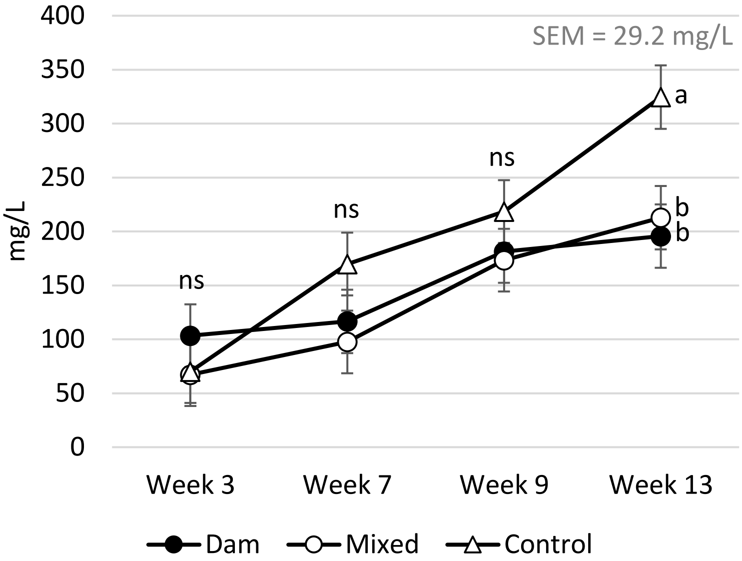


Figure 3.4. Milk lactoferrin concentration (average and standard error) of the three groups of cows by Weeks of lactation.

Health disorders. During the 14 weeks of the trial, the frequency of health disorders was not significantly different between groups of cows and calves (Table 3.6). Only 18% of suckling cows (Dam- and Mixed- cows, on average) attended reproductive disorders, compared to 36% of Control-cows. While 25% of suckling cows, on average, attended non-reproductive disorders compared to 29% of Control-cows. Beside, 18% of suckling calves (Dam- and Mixed-calves, on average) attended respiratory disorders compared to 7% of Control-calves, while 14% of suckling calves attended non-respiratory disorders compared to 29% of Control calves. We concluded that CCC practice did not affect the occurrence of animals' health disorders; nevertheless, we have to consider the low number of animals that do not allow to draw robust conclusions.

Table 3.6. Chi-squared test on the number of cows and calves that attending health disorders in the three groups (Control, Mixed, Dam), during the 14 weeks of the trial. Health disorders are classified into *Reproductive disorders* and *Non-reproductive disorders* for cows, and *Respiratory disorders* and *Non-respiratory disorders* for calves.

			Real			Expected			
			Control	Dam	Mixed	Control	Dam	Mixed	*Chi-squared test*
Cows	Reproductive	yes	5	3	2	3	3	3	*0.40*
		no	9	11	12	11	11	11	
	Non-reproductive	yes	4	3	4	4	4	4	*0.88*
		no	10	11	10	10	10	10	
Calves	Respiratory	yes	1	3	2	2	2	2	*0.53*
		no	8	6	7	7	7	7	
	Non-respiratory	yes	4	1	3	3	3	3	*0.29*
		no	5	8	6	6	6	6	

3.4. Cortisol in the hair of calves

3.4.1. Introduction

Cortisol, also sometimes called the stress hormone, has been increasingly used as a biomarker of stress in animal blood (Romero, 2004). In contrast to cortisol concentration in blood, saliva, faeces, urine and milk, hair cortisol concentration is a marker of chronic stress that reflects a period of few weeks or months (Comin et al., 2011; Meyer and Novak, 2012). As hair cortisol concentration is not likely to be affected by manipulation during sampling and daily physiological or acute changes, hair cortisol seems to be an interesting non-invasive biomarker of chronic stress and has already been associated with housing, management and handling of animals (Heimbürge et al., 2019). However, hair cortisol varies in bovine hair according to a number of fixed factors including age, parity, breed, environment, season, body region and hair colour (Vesel et al., 2020) which makes the definition of thresholds for the qualification of chronic stress difficult. Nevertheless, hair cortisol was successfully related to different stressful conditions including late pregnancy, beginning of lactation, diseases, high stoking density or changes of environment (Vesel et al., 2020). In order to compare the chronic stress level of calves reared or not with their dam until weaning, cortisol measurements in the hair of the calves were performed just before weaning and about 4 weeks after weaning.

3.4.2. Material and Methods

Hair samples were taken at 60.3 ± 4.3 days (before weaning) and about 4 weeks after weaning (29.9 ± 0.3 days). For each sample, a few grams of clean hair was collected from the animal's shoulder using an electric clipper. The individual samples were stored at room temperature in envelopes, protected from light, until the cortisol analysis.
The analysis was carried out at Swedish University of Agricultural Sciences (Department of Clinical Sciences, 750 07 Uppsala, Sweden) using Salimetrics ELISA kit https://salimetrics.com/wp-content/uploads/2018/03/salivary-cortisol-elisa-kit.pdf. The method for preparation of samples and extraction of hair cortisol is presented in the figure below (from Anna Svensson, Laboratory director; personal communication) (Figure 3.5).

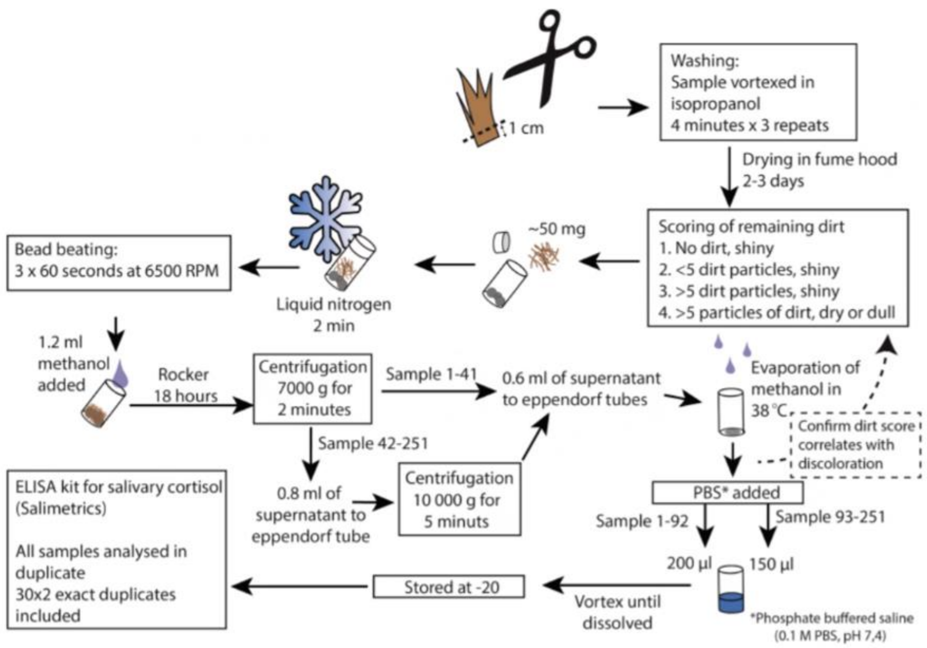


Figure 3.5. Method for preparation of samples and extraction of hair cortisol.

Statistical analyses. After log_{10} transformation and removal of two outliers, the data were analysed by analysis of variance (SAS GLM procedure). The model took into account the rearing practice (Control, Dam, Mixed), the sex (male and female) and the breed of calves (Holstein, Montbéliarde), and for the pre-weaning measurement the age at sampling as a covariate.

3.4.3. Results and discussion

Hair cortisol content measured just before weaning was significantly lower in calves reared with their dam until weaning (Dam group) than in calves separated at birth or after 4 weeks (-26.1% at 19.9 pg/mg; P = 0.017; Table 3.7). This result was obtained even though Dam calves had presumably a higher level of activity as they were grazing with their Dam during the pre-weaning period, contrary to Mixed and Control-calves that stayed inside the barn in their collective pen until weaning. Indeed, change from winter indoor to summer pasture was shown to increase hair cortisol level (Comin et al., 2011; Peric et al., 2017) which reinforces the conclusion that the level of stress of calves reared with their Dam until weaning is lower than the level of chronic stress of calves reared without their Dam.

Four weeks after weaning, the difference found in hair cortisol during the pre-weaning period between groups tended to persist (-27.8% at 11.2 pg/mg; P = 0.080) even though calves reared with their Dam had a stressful abrupt weaning that was confirmed by their earlier and higher vocalizing period, in comparison to control calves at weaning. Considering that the measurement made after weaning reflects the whole period, this trend seems to confirm that the post-weaning period mitigated the differences observed during the pre-weaning period. In addition, female calves had a lower hair cortisol level than male calves after weaning (11 vs 17.4 pg/mg).

Table 3.7. Effect of Group (Dam, Mixed and Control), Breed (Ho and Mo) and Calves' sex (Male, female) on calves' hair cortisol. Hair samples were taken on 9 calves per Group before weaning (60.3 ± 4.3 days) and 3 weeks after weaning (29.9 ± 0.3 days).

	Group				*P-value*		
	Dam	Mixed	Control	SEM	Group	Breed	Sex
Hair cortisol (log10 pg/mg)							
Before weaning	1.30^b	1.42^a	1.44^a	0.036	0.02	0.28	0.28
After weaning	1.05	1.17	1.20	0.051	0.08	0.37	0.01

In conclusion, the passive immunity transfer from cows to calves was adequate in all groups and CCC practices (Dam and Mixed groups) had no adverse effect on neonatal calves' immunity and had no effect on the further build-up of the active immune defense during the pre-weaning period. Moreover, we concluded that the level of chronic stress of calves reared with their Dam until weaning was lower than that of Mixed- and Control-calves.

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CHAPTER 4

Early-life dam-calf contact and grazing experience on post-weaning behavior and herbage selection of dairy calves in the short term

This chapter is based on: [1,2]Nicolao, A., [3]Coppa, M., [4]Bouchon, M., [2]Sturaro, E., [1]Pomiès, D., [1]Martin, B., & [1]Koczura, M. (2020). Early-Life Dam-Calf Contact and Grazing Experience Influence Post-Weaning Behavior and Herbage Selection of Dairy Calves in the Short Term. *Frontiers in Veterinary Science*, *7*(December), 1–11. https://doi.org/10.3389/fvets.2020.600949.

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frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 07 December 2020
doi: 10.3389/fvets.2020.600949

Early-Life Dam-Calf Contact and Grazing Experience Influence Post-Weaning Behavior and Herbage Selection of Dairy Calves in the Short Term

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OPEN ACCESS

Edited by:
Emily Patterson-Kane, Independent Researcher, Rolling Meadows, IL, United States

Reviewed by:
Tony Waterhouse, Scotland's Rural College, United Kingdom
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Specialty section:
This article was submitted to Animal Behavior and Welfare, a section of the journal Frontiers in Veterinary Science

Received: *31 August 2020*
Accepted: *16 November 2020*
Published: *07 December 2020*

Citation:
Nicolao A, Coppa M, Bouchon M, Sturaro E, Pomiès D, Martin B and Koczura M (2020) Early-Life Dam-Calf Contact and Grazing Experience Influence Post-Weaning Behavior and Herbage Selection of Dairy Calves in the Short Term. Front. Vet. Sci. 7:600949. doi: 10.3389/fvets.2020.600949

Rearing dairy calves with their mothers could teach them how to graze, optimizing grass use, and improving their welfare and performance. We tested the short-term effects of dam-calf contact experience on grazing and social behavior of weaned calves, monitored over seven days for their first post-weaning grazing experience. "Dam" (D) calves were reared and grazed with their mothers until weaning. "Mixed" calves (M) were separated from their mothers after 4 ± 0.5 weeks, they experienced dam-calf contact, but not grazing. "Standard" (S) calves had never experienced either dam-calf contact (separated at birth) or grazing. Each group grazed an equivalent pasture plot offering heterogeneous herbage. Scan sampling of calves' activities was performed every 5 min, 6 h per day, on Days 0, 1, 2, 3, and 7. Daily, the time when calves started grazing after introduction to pasture, and the number and duration of their grazing cycles were measured. Daily activities were differentiated into ingestion, rumination, and idling. The proportion of time that calves spent grouped with other individuals or isolated, and standing or lying were recorded. When grazing, their bites were characterized by botanical family group, height of the selected bite and vegetation status. Individual average daily gains from the 2-week periods before and after grazing were calculated, and were equivalent between groups (313 ± 71 g/d). On Day 0, D-calves started grazing immediately (1 ± 4.1 min), unlike M- and S-calves (39 ± 4.1 and 23 ± 4.1 min), and D-calves grazed patches of dry grass 21.7 times less than M-calves and 16.9 times less than S-calves. Dry herbage patch preference and grazing start time differences disappeared on Day 1. Calves spent the same time ingesting and idling, but M-calves spent on average 1.6 times less ruminating than D- or S-calves. The D-calves showed grazing behavior similar to that of adult cows, selecting grasses throughout pasture utilization, although legumes and forbs were present in the grazed layer. On the contrary, M- and S-calves did not express any specific preference. The S-calves spent more time isolated but had more positive reciprocal interactions than the calves in the other groups.

Keywords: grazing behavior, dairy calves, grazing experience, dam-calf contact, post-weaning, social interactions, first grazing

INTRODUCTION

Maximizing production while reducing costs and labor are the main aims of modern dairy systems. This trend often results in an intensification of farming practices, which weakens societal acceptance of dairy production systems (1). Consumers are taking ever greater interest in how their food is produced, and are increasingly aware of environmental issues and animal welfare (2, 3). In dairy production, the most common welfare concerns are the separation of calves from their dams (4) and restricted access to pasture for animals in intensive systems (5). Pasture for dairy cattle offers several advantages for animal welfare and health, such as expression of natural behavior and possible reduction of lameness and claw disorders (6–8) or increased movement with positive effects on longevity (9). Grazing systems also reduce management and feeding costs for the farmer (10, 11). In commercial dairy farms, calves are usually separated from their dams close to birth, and rarely experience grazing during their early lives (12). In France, 60 % of dairy farms use seasonal batch calving during autumn and winter, in order to turn out animals to pasture in the following spring (13). Then, calves and heifers usually graze from spring to autumn, before their first year of age, but only 2% of dairy farms turn out calves to pasture before 6 months of age (13). This strategy allows the synchronization of the peak of herbage growth and the peak of lactation of dairy cows, with fresh herbage covering a large part of their nutritional requirements (14). At the same time, calves have also grown and matured sufficiently and are able to be moved to pasture.

Le Cozler et al. (13) reported that only 4% of farmers keep calves with their dam at later than 24 h, but this practice is increasingly used. Michaud et al. (15) investigated farms using a suckling practice in France (Massif Central, East and West of France), and found that 62 farms out of 102 kept calves with their dam or with a foster cow between 1 and 60 days of age. The presence of the dam in the early stages of a calf's life can have positive effects on its social interactions, feeding behavior, and growth (16–18). The dam is the primary social model and plays an important role in the acquisition of foraging behavior and feed selection (19, 20). Pullin et al. (20) found that lambs grazing with their dam spent more time foraging, were more active, developed long-term feed preferences and learned aversion to toxic feed more effectively than lambs grazing alone. Young animals learn by emulation of social models or by trial and error, although in most cases this last is less efficient (21). Calves usually are neophobic: they tend to choose feed and places they already know, so that individual learning in a new environment takes more time than learning by social models (22, 23). Lopes et al. (24) observed that heifers with early grazing experience, compared to inexperienced heifers, affected grazing behavior and milk production only in the first days on pasture, but showed that the animals would generally adapt to a new environment and a novel feed easily, especially during their first year of life. Dairy calves that have learned to graze with their dam might therefore more efficiently recognize herbage quality and select specific patches when turned out to pasture after weaning, compared to calves that never grazed before. However, it is unclear whether this advantage holds only in the first grazing day or is more persistent.

In the present study, the following hypotheses were tested, comparing three groups of calves with contrasting rearing experience on their first grazing days after weaning. We expected calves that had experienced dam-calf contact and grazing in their early life to show grazing and probably social behavior that was different from that of inexperienced calves, and more typical of adult dairy cows. The longer dam-calf contact lasted (a few weeks or until weaning), the greater would be the expected differences in calves' social behavior. The present study also evaluated the persistence of the expected differences in grazing or social behavior in the short term after weaning.

MATERIALS AND METHODS

Experimental Design

The experiment was performed in 2019 at the INRAE experimental farm of Marcenat (DOI: https://doi.org/10.15454/1.5572318050509348E12), located in the Massif Central (45°15′N, 2°55′E; 1150 m a.s.l.). All animal-related procedures were carried out in accordance with the guidelines for animal research of the French Ministry of Agriculture and all other applicable national and European regulations for experimentation with animals (https://www.recherche-animale.org/sites/default/files/charte_nationale_portant_sur_l_ethique_de_l_experimentation_animale_243579.pdf). The experiment started February 12. The early grazing period started July 22 and ended July 29. Three breed-balanced groups of eight dairy calves (Holstein and Montbéliarde) with different experience backgrounds were compared (**Table 1**): a group of "Standard" calves (S) that had been separated at birth from their dam and had never experienced grazing, a group of "Dam" calves (D) that had been reared and grazed with their dam until weaning, and a group of "Mixed" calves (M) that had been separated from their dam at 4 ± 0.5 weeks of age and had never experienced grazing. All calves were weaned at age of 10.9 ± 1.1 weeks. Before weaning, D-calves were housed separately from their dams at night and had free access to the dam cowshed during the day. Starting from May 5, when the calves were 4.6 ± 3.2 weeks old, the day cowshed access was replaced by free access to pasture with dams. The M-calves, until age 4.0 ± 0.5 weeks, were reared in the same way, except that they had no access to pasture. From this age until weaning, they were reared like S-calves, i.e., in separate housing and fed bulk milk with an automatic milk dispenser. D- and M-calves were reunited with their dams after morning milking at 9:00 a.m. and separated before evening milking at 3:30 p.m. At weaning, all calves were moved to a new pen, with one pen for each group to prevent mixing. In this pen, calves ingested 0.5 kg/d/calf of hay and 2.0 kg/d/calf of concentrate (Startivo, Centraliment, 15006 Aurillac). Hay was distributed in the evening with no refusal left in the morning. Concentrate was distributed half in the morning and half in the evening, until the end of the study. After the last weaning, all calves spent at least six days indoors all together to allow the latest weaned calves to adapt to the new conditions. At the beginning of the grazing period (week 15), D-, M- and S-calves were, respectively, 14.9 ± 3.2, 16.1 ± 2.8 and 15.3 ± 3.6 weeks old and weighed 131 ± 18.3 kg, 123 ± 17.4 kg, and

TABLE 1 | Feeding plan (milk, concentrate, and hay) of the three groups of calves (Standard, Dam, Mixed) during the first 15 weeks of age.

Group	Week	1	2	3	4	5	6	7	8	9	10	11..	15
Standard	Milk[1] (kg/d)	6.0	7.0	9.0	10.0	10.0	10.0	9.0	7.0	5.0	3.0	Weaning	Start grazing
	Concentrate[2] (kg/d)	0	0	0.2	0.4	0.6	0.9	1.2	1.5	1.8	2.0	2.0	2.0
	Hay[3]	0					*ad libitum*					*ad libitum*	0.5
Dam	Suckling period	24 h/24 h					Between morning and evening milkings (=during the day)					Weaning	Start grazing
	Concentrate (kg/d)	0					*ad libitum*					2.0	2.0
	Hay	0					*ad libitum*					*ad libitum*	0.5
	Pasture with dams	/		/					*During the day*			/	
Mixed	Suckling period	24 h/24 h	During the day		10.0	10.0	10.0	9.0	7.0	5.0	3.0	Weaning	Start grazing
	Concentrate (kg/d)	0	*ad libitum*		0.4	0.6	0.9	1.2	1.5	1.8	2.0	2.0	2.0
	Hay	0	*ad libitum*						*ad libitum*			*ad libitum*	0.5

[1] *bulk milk distributed individually by automatic feeder.*
[2] *first age concentrate distributed individually by automatic feeder (Standard group and Mixed group after separation from the dam) or in collective bucket (Dam group and Mixed group before separation from the dam).*
[3] *permanent grassland hay (first cut) distributed in a rack.*

TABLE 2 | Characteristics of vegetation offered on the experimental plots (mean ± standard deviation).

Plot characteristics	Dam	Mixed	Standard
Patch type (%) and description			
Dry (≥70% dead material)	15.5 ± 4.1	16.0 ± 2.1	13.3 ± 2.5
Green (< 70% dead material)	84.5 ± 7.2	84.0 ± 4.1	86.7 ± 6.0
Grasses (≥ 70% grasses)	65.1 ± 9.7	69.1 ± 6.6	64.2 ± 7.3
Legumes (≥ 30% legumes)	17.1 ± 4.2	13.6 ± 2.0	13.3 ± 3.4
Forbs (≥ 30% forbs)	17.8 ± 3.6	17.3 ± 1.4	22.5 ±4.8
Tall (≥ 25 cm)	51.2 ± 7.8	48.1 ± 4.5	53.3 ± 7.6
Intermediate (7 cm ≤ x < 25 cm)	33.3 ± 5.7	35.8 ± 4.1	32.5 ± 5.1
Short (< 7 cm)	15.5 ± 5.2	16.0 ± 2.6	14.2 ± 3.1
Composition and nutritional value			
Dry matter *(g/kg),*	32.5 ± 3.3	28.3 ± 5.9	31.6 ± 2.5
Organic matter digestibility *(g/kg DM)*	67.2 ± 2.8	67.0 ± 3.8	66.0 ± 1.7
NDF *(g/kg DM)*	53.9 ± 4.4	53.8 ± 1.2	53.9 ± 1.8
ADF *(g/kg DM)*	27.6 ± 1.6	28.2 ± 1.0	28.5 ±1.9
Crude protein *(g/kg DM)*	12.0 ± 1.6	12.2 ± 2.7	10.6 ±1.8

128 ± 23.5 kg respectively, on average. They had been weaned for 30 ± 22, 33 ± 20 and 33 ± 24 days, respectively. Calves were turned out to pasture on July 22 (Day 0), from 9:00 a.m. to 5:00 p.m.

Characteristics of the Experimental Plot

Calves grazed a permanent pasture divided into three equivalent neighboring 0.15 ha plots. No close visual contact was allowed between the three groups of animals, by fencing the plots so that they were at least 15 m apart. To encourage grazing selection for all three groups of calves, the whole plot was strip-mown 28 days before the grazing period started. At Day 0, plots were thereby composed of alternate 3 m strips dominated by mature vegetation and vegetative regrowth. The botanical composition of the whole pasture was determined using the vertical point-quadrat method from Daget and Poissonet (25). The pasture was dominated by *Lolium perenne* (39.0%), *Agrostis tenuis* (15.0%), and *Trifolium repens* (13.5%). *Rumex obtusifolius* was also present (3.1%). At the beginning of the experiment, three 10 cm × 3 m grass samples were collected on each plot, perpendicularly to the mown and unmown strips, equally harvesting the same length from both. They were oven-dried at 60°C for 72 h and analyzed for proximate composition as described by Coppa et al. (26) (**Table 2**).

Observations and Behavior Measurements

Calves were weighed once a week, and individual average daily gain (ADG) from the 2-week periods before and after the grazing period started was calculated.

Individual daily activities and behavior were observed by scan sampling at 5-min intervals (27, 28) on the day the calves encountered the pasture for the first time (Day 0), the next three days (Day 1, Day 2, Day 3) and one week later (Day 7), focusing on the first days as most of the differences were expected here (24). On each plot, four calves were randomly assigned to two observers for 6 h per day (9:00–12:00 a.m. and 2:00–5:00 p.m.). For observations, calves were always identified by the same numbers painted on their back. At the end of the afternoon, the calves went back indoors for the night where they were fed with hay and concentrate (Startivo, Centraliment, 15006 Aurillac). Observers, randomly assigned to a group of calves, changed experimental group between each morning and afternoon. Each day, the time taken by calves to start grazing was measured. A calf was considered to have started grazing if it was observed taking a bite in at least three out of four successive observations (29), following the flowchart in **Figure 1**. From the time the calf started grazing, the grazing cycle lasted until it showed more than three other successive activities (i.e., it stopped grazing for at least 15 min), according to Manzocchi et al. (30). The duration of a grazing cycle and the number of grazing cycles, as just described, were calculated following the flowchart in **Figure 2**.

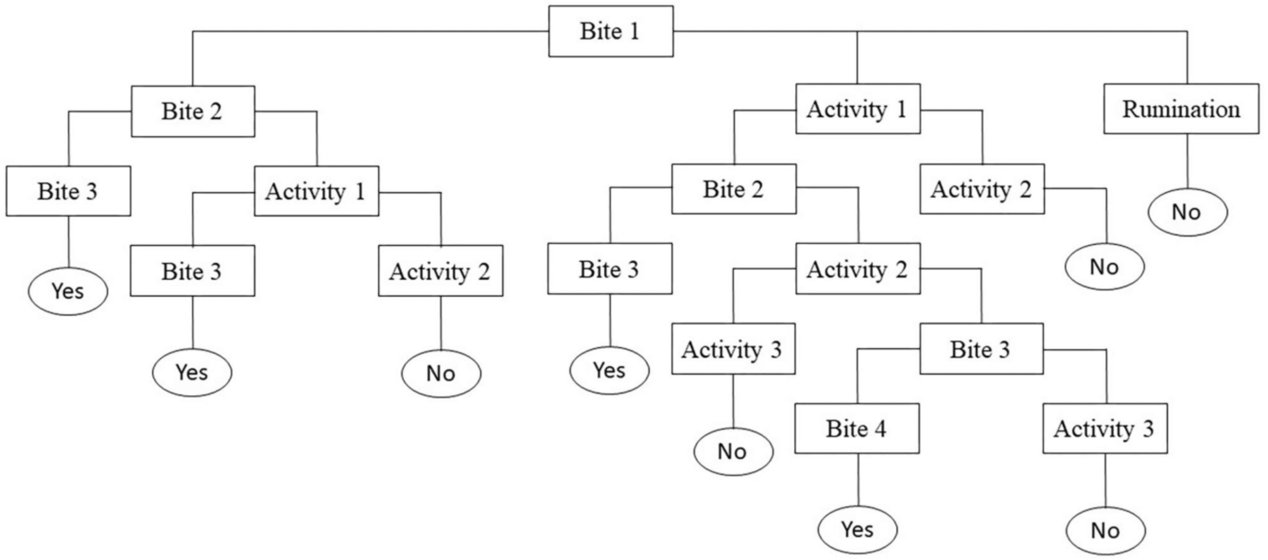

FIGURE 1 | Flowchart of the conceptual scheme used during observations to tell whether calves started grazing at Bite 1 (Yes = at least three bites over four observations; No = flowchart restart to the next observation).

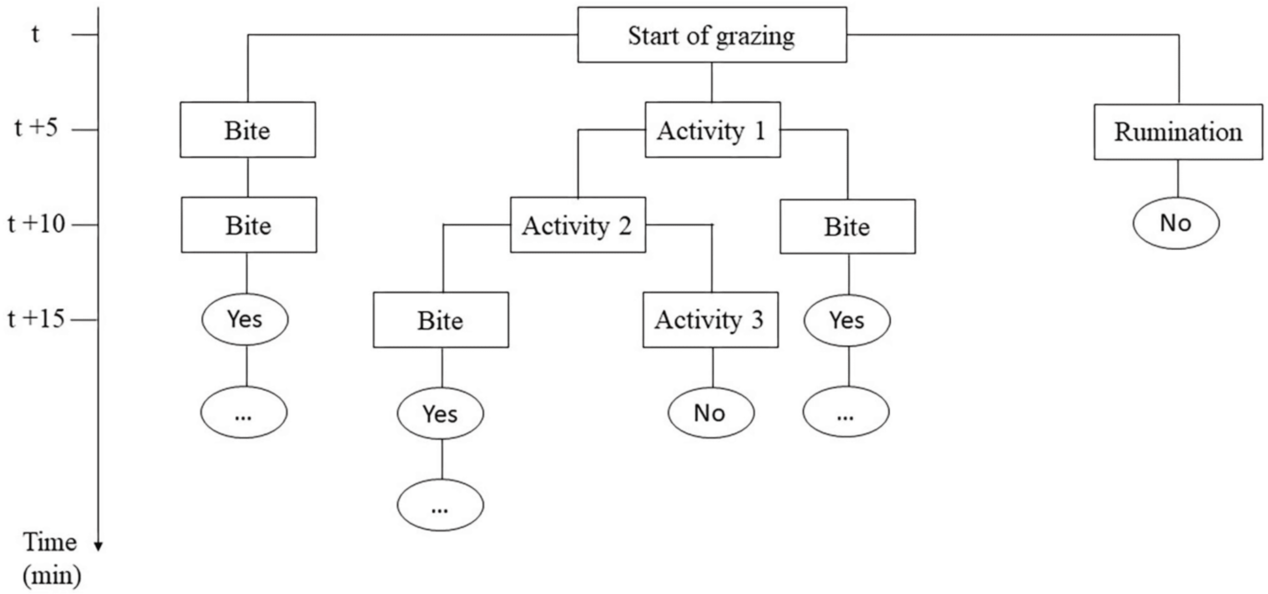

FIGURE 2 | Flowchart of the conceptual scheme used during observations to tell whether a grazing cycle was established (Yes or No) and measure its duration.

Daily activities were then differentiated into three groups: ingestion (grazing and drinking water), rumination, and idling. The latter comprised four subcategories: resting (observation, sleep, self-grooming), positive interactions (licking, sniffing, head play), negative interactions (head-butting, pushing, fighting) and *ad hoc* activities (walking, exploring, stereotypies, vocalizing) (**Table 3**). The daily proportion of ingestion, rumination and idling time was calculated as a percentage of the total daily observations. The daily proportion of resting time, socializing time and *ad hoc* activities was calculated as a percentage of the idling activities. Each time one of the activities was recorded, observers also indicated whether the calf was grouped with other calves or isolated, i.e., at least 3 m away from other calves, and whether it was standing or lying. The daily proportions of time spent grouped and standing were calculated over the total number of observations of the day. When calves

TABLE 3 | Description of daily activities recorded by scan-sampling differentiated in four subcategories.

Daily activity	Subcategory	Behavior type	Description
Ingestion			*Grazing and drinking water*
Rumination			*Ruminating*
Idling	Resting	Observation	*Standing or lying, without sleeping*
		Sleep	*Sleeping*
		Self-grooming	*Self-licking, rubbing, defecating and urinating*
	Positive interaction	Licking	*Licking another calf's head or body*
		Sniffing	*Sniffing another calf's head or body*
		Head play	*Rubbing the head against the head of another calf*
	Negative interaction	Head-butting	*Pushing the head against the head of another calf*
		Pushing	*Pushing the head against the body of another calf*
		Fighting	*Two calves pushing each other*
	Ad hoc activities	Walking	*Walking*
		Exploring	*Sniffing the floor, sniffing/ licking objects, discovering the environment*
		Stereotypies	*Cross-suckling, tongue rolling and repeatedly sniffing/licking objects*
		Vocalizing	*Mooing punctually and/or repeated*

were grazing, their bites were characterized by botanical group (grasses, legumes and forbs), the height of the selected bite (tall, intermediate, short vegetation) and the vegetation status ("dry" or "green"), according to Koczura et al. (31). Briefly, patches were characterized according to the visually estimated proportion of dry senescent herbage, of botanical family groups and of their height (26, 32). A patch was coded as "dry" if the dry senescent vegetation represented more than 70 % of the bite, as "green" if it was $< 70\%$; as dominated by "grasses" if the bite contained more than 70% of grasses, by legumes or forbs if they represented more than 30%; tall if herbage height was ≥ 25 cm, and small if it was ≤ 7 cm, as detailed in **Table 2**. Observers were able to get close to calves due to their adaptation to human presence achieved during the pre-weaning experiment. When calves ingested forbs, observers reported whether or not they selected *Rumex* thanks to a binary variable (1 = the calf tried to eat *Rumex* at least one time in the observation day). The daily proportion of vegetation type ingested by calves was calculated as a percentage of observations comprising the vegetation type compared to the total number of grazing observations of the day.

The weather was exceptionally hot on the afternoons of Day 2 and Day 3. The average maximum daily temperature during these afternoons was 31.2°C, whereas between 2000 and 2019, the average maximum temperature in July was 21.6°C (INRAE CLIMATIK 2.1.5, Marcenat weather station). Behavior observations at pasture were therefore made throughout the day on Day 0, Day 1, and Day 7, but only in the morning on Day 2 and Day 3. The daily ingestion, rumination and idling activities, together with the number and duration of grazing cycles, were accordingly calculated only for Day 0, Day 1, and Day 7, as the morning alone was not considered representative of the ingestion and rumination cycles of a whole day. On the other hand, the characterization of grazed bites and time needed to start grazing were calculated on mornings only for all days, the numbers of bites observed during the morning being considered sufficient and representative to express preference, as differences between morning and afternoon on those days were equivalent.

Statistical Analysis

Daily activities and grazing cycles were analyzed with a repeated MIXED model on SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). Group (Dam, Mixed or Standard), day (only 0, 1, or 7) and their interaction were included as fixed effects. The individual calf was considered as the subject of repetition, with day being the repeated factor. We used a compound symmetry covariance structure. Time to start grazing and herbage selection were analyzed with the same model, except that the day effect included all days. Average daily gain was analyzed with a similar repeated model, which included group, period (before or after pasture) and their interaction as fixed effects, calf as subject, and period as repeated factor. In this last model, the number of days since each calf had been weaned was used as a covariate. The effect of the age and BW of calves were tested as covariates as well, but were found to be non-significant, and so were not finally included in the model. For all data, normality of residuals was checked using the Shapiro-Wilk test. The frequency of times calves tried at least 1 time to include *Rumex* in their bites was compared between groups using a Chi^2 test. Significance was set at $p < 0.05$.

RESULTS

ADG Before and After Grazing

During the 2 weeks before start of grazing, the ADG of D-, M- and S-calves did not differ significantly ($p = 0.177$), at 285, 355, and 480 g/d, respectively. During the following 2 weeks it increased by 313 g/d on average for all the groups.

TABLE 4 | Effect of early dam-calf contact and grazing experience on post-weaning daily activities and grazing cycles of dairy calves (Day 0, 1 and 7 after start of grazing).

Item	Dam	Mixed	Standard	SEM	Group	Day	Group × day
Daily activities (% of daily total observations)							
Ingestion time	55.8	58.3	58.2	1.88	ns	**	†
Rumination time	11.0^{a}	6.9^{b}	10.2^{a}	1.01	*	*	ns
Idling time	33.2	34.8	31.7	1.88	ns	***	ns
Grazing cycles (by day)							
Duration (min)	57.8	55.9	55.6	4.97	ns	†	ns
Number	3.1	3.5	3.2	0.21	ns	*	ns
Idling activities (% of daily idling observations)							
Resting time[1]	64.6^{a}	55.4^{b}	56.5^{b}	2.55	*	***	ns
Ad hoc activities[2]	30.2^{b}	37.3^{a}	35.4^{ab}	1.89	*	***	ns
Positive interactions[3]	1.6^{b}	0.7^{b}	3.3^{a}	0.51	**	ns	ns
Negative interactions[4]	3.7	6.5	4.8	1.07	ns	**	ns
Proportion of time (% of daily observations) spent:							
Lying	15.8^{b}	9.8^{c}	20.5^{a}	1.60	***	***	***
Isolated	22.3^{b}	19.1^{b}	31.6^{a}	2.07	***	**	***

***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; †$p < 0.10$; ns $p \geq 0.10$.
$^{a-c}$Means within a variable with different superscript letters differ at $p < 0.05$.
[1] Resting time: observation, sleeping, self-grooming.
[2] Ad hoc activities: walking, exploring, stereotypies, vocalizing.
[3] Positive interaction: licking, sniffing, head play.
[4] Negative interaction: head-butting, pushing, fighting.

Effect of Early Dam-Calf Contact and Grazing Experience on Calves' Daily Activities

Once turned out to pasture, the daily activities of the three groups of weaned dairy calves did not strongly differ (interaction groups × day non-significant). Overall, during Days 0, 1, and 7, calves in the three groups spent almost the same time ingesting ($p = 0.081$, on Day 1 M-calves tended to spend less time ingesting than D- and S-calves) and the same time idling (**Table 4**), but M-calves spent on average less time ruminating than D- and S-calves (1.54 times less). During idling activities, D-calves spent more time resting than M- or S-calves (1.16 and 1.14 times more, respectively), and M-calves spent more time in *ad hoc* activities than D-calves (1.24 times more). The S-calves had more positive social interactions than the calves in the other two groups. On Day 0 and Day 1, S-calves spent more time lying than D- or M-calves (**Figure 3I**). On Day 0 and Day 1 they spent more time isolated than calves in the other two groups (**Figure 3II**).

Effect of Early Dam-Calf Contact and Grazing Experience on Calves' Herbage Selection

When moved to pasture, D-calves started grazing immediately (**Table 5**), whereas it took S-calves 23 ± 4.1 min to actively start to graze. The M-calves needed a further 20 min. On Day 0, the herbage selection was different between groups: D-calves grazed "dry" patches 21.71 times less than M-calves and 16.90 times less than S-calves. On Day 0, no differences between groups were observed for botanical composition and height, except for forbs: on that day, S-calves grazed 13.73 times more forbs than M-calves and 3.89 times more than D-calves. On Day 1, Day 3, and Day 7 all three groups of calves started grazing 5 ± 2.8 min after arriving on pasture, whereas M- and S-calves started grazing 15 ± 0.7 min after D-calves on Day 2. From Day 1, M- and S-calves reduced their proportion of "dry" patches to meet that of D-calves, with no longer any significant differences between groups. Overall, we found that the proportion of tall vegetation in the bites decreased from Day 1 to Day 7 and conversely that the proportion of short vegetation increased in the bites from Day 1 to Day 7. On Day 2 and Day 3, M-calves showed a higher proportion of intermediate vegetation than D- and S-calves (2.07 and 3.03 times more, on average). D-calves continuously maintained stable the proportion of grasses in their bites throughout the plot utilization, while M- and S-calves decreased their proportion over time (0.75 times less from Day 0 to Day 7, on average), increasing in parallel those of legumes and forbs (7.57 and 4.46 more times on average, respectively). On Day 0, none of D-calves grazed *Rumex,* on the contrary to M- and S-calves (4 and 6 calves, respectively) (**Figure 4**). This difference disappeared in the following days, already on Day 1.

DISCUSSION

Effect of Early Dam-Calf Contact and Grazing Experience on Calf Grazing Behavior

To our knowledge, only a few published studies have focused on dairy calf grazing behavior, and this is the first time that the effects of an early dam-calf contact have been investigated on calf grazing behavior, directly after weaning. As expected, the

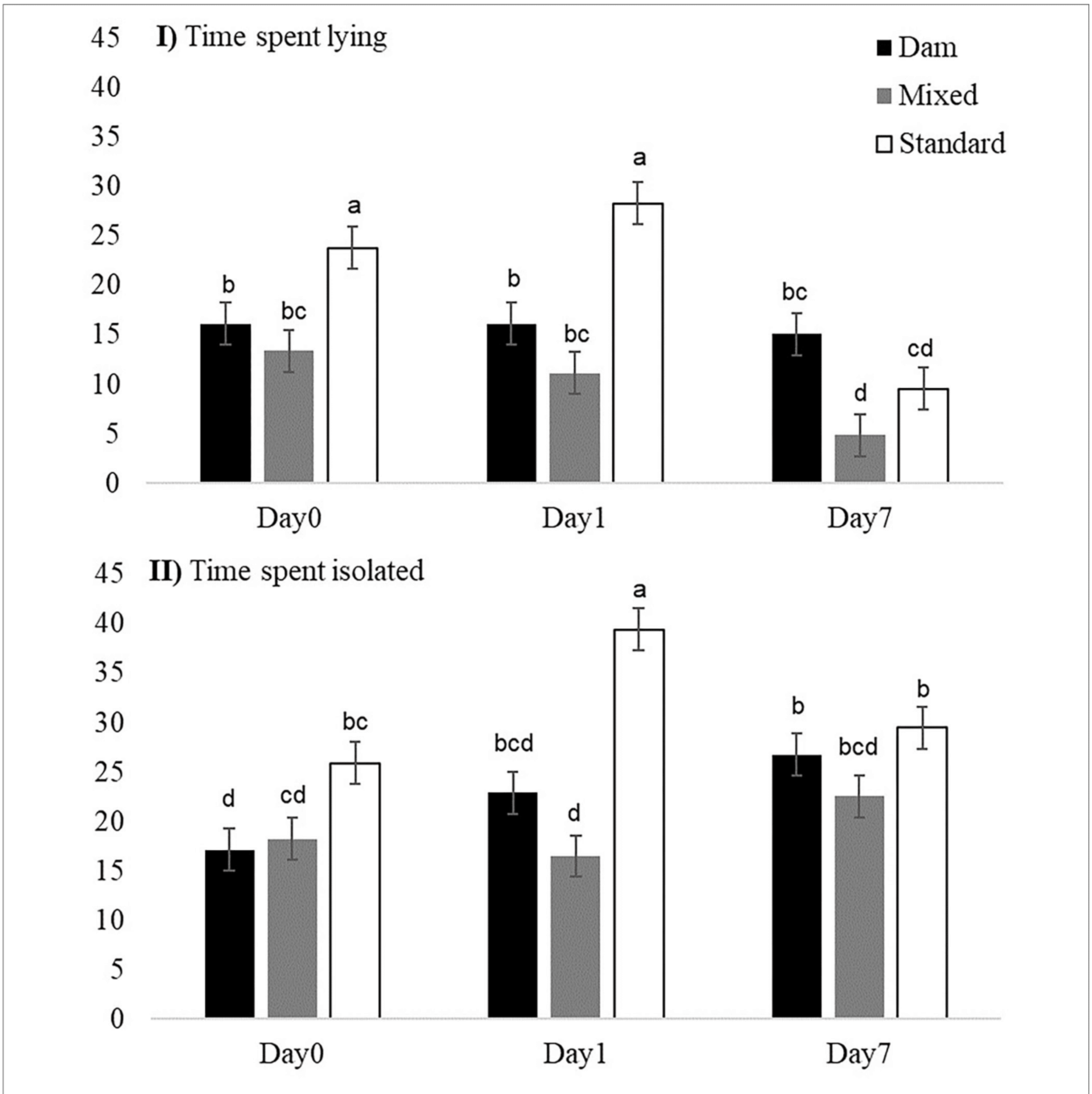

FIGURE 3 | Effect of early dam-calf contact and grazing experience on **(I)** daily time spent lying (%) and **(II)** isolated (%) by calves on Day 0, Day 1, and Day 7 at pasture. Bars are standard errors. $^{a-d}$Means within a variable with different superscript letters differ at $p < 0.05$.

main differences in calf grazing behavior were mostly observed on the first day on pasture (Day 0): D-calves started grazing immediately when moved to pasture, whereas S- and M-calves started 23 and 43 min later, respectively. In several studies, it is reported that inexperienced heifers need a few hours (12) or a few days (24) to start grazing. This lag occurs even when animals are put on pasture with experienced heifers. In our study, the three groups were separated to prevent visual contact between experienced and non-experienced animals. Calves that had experienced pasture with dams in their early life then immediately remembered how to graze, unlike calves from the other groups. This is consistent with findings of Lopes et al. (24), who observed that heifers that had once experienced pasture instantly remembered how to graze the following year. The M- and S-calves took slightly longer to start to graze, probably because they had no social model or experienced individuals

TABLE 5 | Effect of early dam-calf contact and grazing experience on time to start grazing after introduction to pasture and characteristics of selected bites by dairy calves.

Item	Group	Day					SEM	Group	Day	Group × day
		0	1	2	3	7				
Time to start grazing (min)	Dam	1^{e}	6^{e}	3^{e}	2^{e}	4^{e}	4.13	***	***	***
	Mixed	39^{a}	4^{e}	18^{bcd}	11^{cde}	7de				
	Standard	23^{b}	4^{e}	19^{bc}	6^{e}	2^{e}				
Herbage selection (% of ingestion observations)										
Green	Dam	97.9^{a}	100^{a}	97.2^{a}	100^{a}	98.1^{a}	0.03	***	***	***
	Mixed	54.5^{c}	98.0^{a}	99.2^{a}	98.5^{a}	95.2^{a}				
	Standard	64.5^{b}	94.6^{a}	92.8^{a}	98.3^{a}	99.5^{a}				
Dry	Dam	2.1^{c}	0^{c}	2.8^{c}	0^{c}	1.9^{c}	0.03	***	***	***
	Mixed	45.6^{a}	2.0^{c}	0.8^{c}	1.5^{c}	4.8^{c}				
	Standard	35.5^{b}	5.4^{c}	7.2^{c}	1.7^{c}	0.5^{c}				
Grasses	Dam	86.4^{abc}	75.6^{cd}	81.3^{abc}	91.9^{a}	87.1^{abc}	0.04	***	***	***
	Mixed	87.8^{ab}	86.8^{abc}	59.5^{ef}	52.7^{f}	66.6^{de}				
	Standard	77.8^{bcd}	84.3^{abc}	61.8^{ef}	53.1^{f}	58.3^{ef}				
Legumes	Dam	8.3^{def}	15.6^{bcd}	11.3^{cdef}	4.9^{ef}	7.1^{def}	0.03	***	***	***
	Mixed	10.7^{def}	11.1^{def}	39.6^{a}	40.5^{a}	21.6^{b}				
	Standard	1.60^{f}	4.0^{f}	14.9^{bcd}	13.4^{bcde}	21.0^{bc}				
Forbs	Dam	5.3^{cde}	8.8^{cd}	7.3^{cde}	3.2^{de}	5.9^{cde}	0.03	***	**	***
	Mixed	1.5^{de}	2.1^{de}	0.8^{e}	6.8^{cde}	11.9^{c}				
	Standard	20.6^{b}	11.8^{c}	23.3^{b}	33.5^{a}	20.7^{b}				
Tall	Dam	84.3^{ab}	61.7^{efg}	68.0^{cdef}	76.8^{abcd}	50.2^{g}	0.06	ns	***	***
	Mixed	73.8^{abcdef}	83.5^{abc}	61.3^{defg}	49.9^{g}	52.2^{g}				
	Standard	76.1^{bcde}	89.2^{a}	77.7^{abc}	77.5^{abc}	58.6^{fg}				
Intermediate	Dam	7.0^{g}	19.1^{cde}	18.1^{cdef}	11.9^{efg}	32.3^{ab}	0.04	***	***	*
	Mixed	14.0^{defg}	11.9^{efg}	34.8^{ab}	26.4^{bc}	41.5^{a}				
	Standard	7.1^{fg}	6.8^{fg}	8.2^{efg}	14.5^{defg}	23.8^{bcd}				
Short	Dam	6.7^{cd}	10.4^{bcd}	7.8^{cd}	7.1^{cd}	15.6^{ab}	0.03	ns	**	***
	Mixed	7.4^{bcd}	1.7^{d}	3.9^{d}	22.0^{a}	6.3^{cd}				
	Standard	2.9^{d}	3.4^{d}	13.4^{abc}	5.9^{d}	13.5^{abc}				

$^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$; $+ p < 0.10$; ns $p \geq 0.10$.
$^{a-g}$Means within a variable with different superscript letters differ at $p < 0.05$.

to emulate (33, 34). Also, inexperienced heifers spend more time exploring and tasting grass than ingesting it, compared to experienced heifers (33), which was numerically the case for our M- and S-calves here before they started grazing, even though exploring time was not long enough to statistically analyze it (data not shown). After starting grazing (on Day 0), M- and S-calves selected mainly "dry" patches, unlike D-calves, which directly grazed only "green" ones. This suggests that inexperienced calves could be neophobic (22): they were probably reluctant to try novel feed, and without a social model, were inclined to choose feed they already knew, or with similar characteristics to hay. Nevertheless, these differences were no longer seen in the following days, showing that calves can soon learn how to graze and cope with novelty.

Once they started grazing, all the calves followed the same pattern from Day 0 to Day 7: they first selected tall vegetation and then intermediate and short herbage as pasture utilization progressed. This is consistent with the selection of vegetation by stratum by experienced grazing cows under rotational grazing (35): once the upper layer is grazed, the height of the patch decreases, moving down to the lower layers (26). However, the botanical selection of the vegetation seemed different between groups: M- and S-calves ingested mainly grasses during the first days, as grasses are almost exclusive in the top layer, and then increasingly legumes and forbs [present in the intermediate and low layers, because of their smaller size; (34)]. On the contrary, D-calves constantly selected grasses until the seventh day of observation, whatever the height of the layer present on the plot. This suggests that inexperienced calves did not select vegetation according to its botanical composition, but rather ingested species according to their presence in the topmost layer as they utilized the plot. Calves that had experienced pasture with dams seem to have learnt to graze like adult cows, which are known to select grasses even on biodiverse pasture (26, 35). Furthermore, M- and S-calves tried to ingest *Rumex*, especially during the first day at pasture, while D-calves rarely approached it. *Rumex*

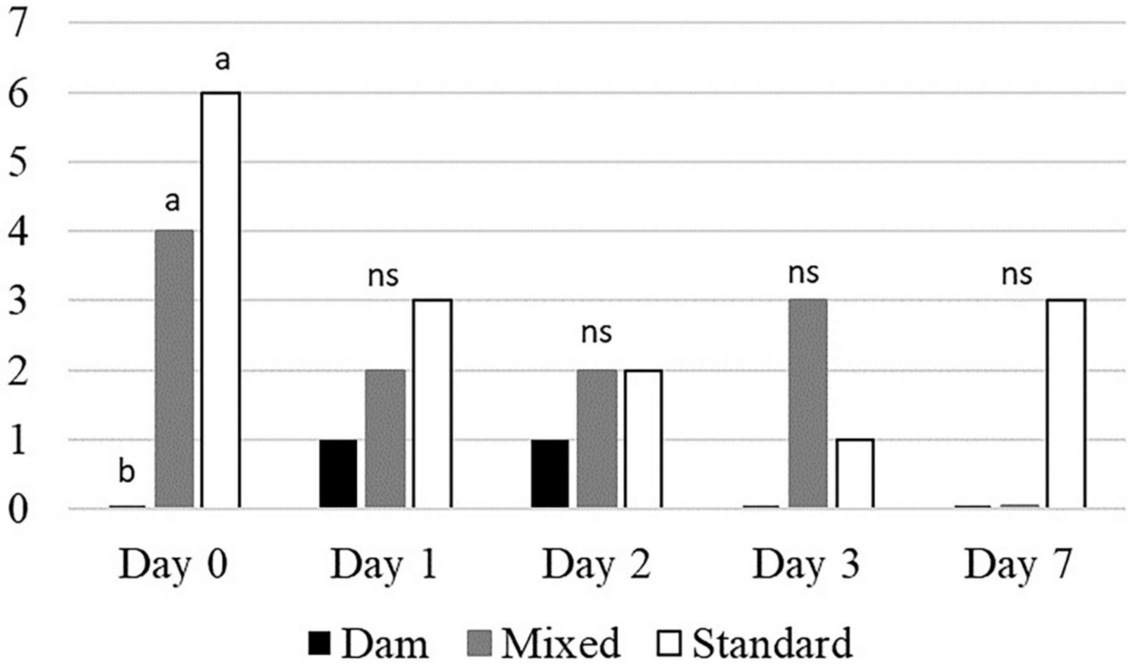

FIGURE 4 | Overall number of calves that tasted *Rumex* each day ($n = 8$ calves × 3 groups). [a–b]Means with different superscript differ at $p < 0.05$, Khi² test; *ns*, not significant.

is one of the main oxalate-producing plants: oxalate can cause poisoning in livestock if present in 10% or more of the dry weight of the plant (36). It is therefore important that cattle learn how to avoid it. This suggests again that calves that have grazed with their dams learnt to choose or avoid some plants (20), while inexperienced calves learnt by trial and error (21). Even though calves that did not experience grazing showed different grazing behavior than D-calves on the first day at pasture, their behavior evolved very quickly (less than a week) into behavior similar to adult cows. This implies that dam-calf contact close to birth has little impact on longer term grazing behavior.

Having experienced dam contact and/or pasture affected the time to start grazing and herbage selection behavior of dairy calves, but did not influence their daily ingestion time or the duration and number of their grazing cycles. All the groups of calves, regardless of their different previous experience, had the same grazing rhythm throughout the trial: this confirms that inexperienced animals exhibit similar grazing times to experienced animals, as found by Lopes et al. (24) and Hessle et al. (19). While idling, M-calves behaved differently from D-calves in *ad hoc* activities (i.e., walking, exploring, stereotypies and vocalizing). This was consistent with the finding of Arrazola et al. (33) highlighting that inexperienced calves spent more time walking and exploring compared to experienced calves, that spent more time inactive. Besides, M-calves spent less time lying than the calves in the other groups. Wilcox et al. (37) demonstrated that standing behavior could indicate a stress condition of the calves, especially in case of chronic stress. Even if we did not directly measure stress of the calves, it could not be excluded that repeating stress factors over time by splitting separation and weaning could have induced a stressful behavior for M-calves. We also found that M-calves spent less time ruminating than D-calves, while the latter spent more time resting. As rumination time is proportional to forage intake, this result suggests that although the ingestion time was similar between groups, M-calves may have ingested less forage than D-calves, as found by Arrazola et al. (33). However, the calves' daily forage intake was not monitored in the present study. A different digestibility of dry senescent and vegetative or tall and short patches (leaf to stem ratio) could also have affected rumination time, but the day by day differences among groups in patches characteristics are not consistent with the trend observed in rumination time. Furthermore, no differences in ADG between groups were observed before and after calves started grazing, even though in the literature inexperienced grazers were found to be nutritionally disadvantaged because of modest foraging behavior that could affect their live weight gains (19). This suggests that the calf daily forage intake was not different between groups. We cannot therefore confirm that the foraging skills of inexperienced calves were inferior, but we can assert that they were not typical of an adult cow.

Effect of Early Dam-Calf Contact and Grazing Experience on Calves' Social Behavior

To the best of our knowledge, this is the first study to investigate dam-calf contact effects after weaning. Valníčková et al. (38) did not find any effect of dam-calf contact on social interactions or play behavior during colostrum feeding. Le Neindre and Sourd

(39) found that heifers reared with foster cows dominated more than heifers reared without cow contact. We thus expected that calves reared with their dams would be more sociable or have more dominant behaviors than artificially reared ones, but we found no differences in negative interactions (i.e., dominance behaviors, such as head-butting, pushing, or fighting) between groups at pasture. Nevertheless, we observed that S-calves had more positive interactions with their companions (particularly licking) than did calves in the other two groups. Pinheiro Machado et al. (40) found that licking behavior between grazing dairy cows was not a random choice but showed a companion's preference for socio-positive interactions. Furthermore, they observed that licking was more persistent in long-established social groups. This could suggest that D- and M-calves may have created bonds rather with dams than with other calves, compared to S-calves, but this point requires further investigation. Besides, the higher proportion of time spent isolated by S-calves, compared to D- and M-calves, could suggest that they exhibit less gregarious behavior. It is however difficult to interpret, because of a lack of literature on this topic.

CONCLUSION

Early life experience with dam and/or pasture influenced calves' foraging skills in the short term after weaning, especially on the first grazing day. Calves that had already experienced pasture with their dams immediately started to graze the day they were turned out to pasture in groups after being weaned. They instantly selected "green" patches of vegetation while grazing, unlike calves that had been housed indoors the whole time, which ingested predominantly senescent herbage on their first day. Daily ingestion time and duration and number of grazing cycles were not affected by previous experience. Nevertheless, botanical selection throughout pasture utilization and rejection of toxic plants (*Rumex*) showed that young calves could already exhibit post-weaning grazing behavior similar to that of adult cows when put on pasture early with their dam. This study provides evidence that separation of dairy calves from their dams close to birth has little impact on grazing behavior, as they grazed similarly to adult cows already in the short term (less than a week after being introduced to pasture). We spotted some differences in social behavior between the calves that experienced dam-calf contact and those that did not, but these differences are not easy to interpret and should be investigated in future studies. Further investigation is also needed to evaluate whether an early grazing experience with their dams could provide positive effects on behavior in the long term and performance in the future lactating careers of these calves.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because the experiment was performed at Marcenat, INRAE experimental farm (Certificate of Authorization to Experiment on Living Animals No. D 15-114-01). No ethical approval was required because defined severity level was 0.

AUTHOR CONTRIBUTIONS

AN, MK, MC, MB, DP, and BM contributed to the conception and design of the study. AN, MK, MC, DP, and BM participated in the collection of behavioral observations data. AN and MK did the data curation and treatment. MK performed the statistical analysis. AN and MK wrote the first draft of the manuscript. MK and MC supervised the experiment and the writing of the manuscript. All authors contributed to manuscript revision, and all read and approved the submitted version.

FUNDING

This study was part of the EU-project ProYoungStock funded by CORE Organic Cofund funding bodies, which are partners of the Horizon 2020 ERA-Net project CORE Organic Cofund. This research was financed by the French government IDEX-ISITE initiative 16-IDEX-0001 (CAP 20-25). AN was supported by a doctoral fellowship from the Fondazione Cariparo at University of Padova (Italy).

ACKNOWLEDGMENTS

The authors thank the staff of the INRAE farm at Marcenat (UE Herbipôle) for animal care, Anna Mathieu for her active participation in the trial, Nadège Aigueperse for advice on experimental design, and Cyril Labonne, André Guittard and Vanessa Conte for their participation in the collection of behavioral observations data.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

CHAPTER 5

Suckling of dairy calves by their dams: trade-off between societal demand and economic sustainability for farmers

This Chapter presents the results of the economic simulation on the impact of two suckling practices (*Short-time contact* and *Day-time contact*) on three different farming systems in France. Preliminary results were presented at the 24th Congress of Animal Science and Production Association held in Padova (Italy) on the 21st-24th of September 2021.

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Abstract

Early separation of dairy calves from their dams is a traditional practice, which simplifies the work of farmers. However, a more ethical rearing system for female calves, allowing cow-calf contact (CCC) until weaning is requested by consumers and should be tested in terms of farm profitability. The aim of this study was therefore to simulate, using a database, the impact of some female calves CCC rearing practices on the economic performance of intensive, extensive and organic dairy farming systems, in France. From experimentations on various CCC rearing practices set up an INRAE experimental farm (2017-2019), we selected the technical results (milk sold, milk composition, calves growth, workload) of a *Short-time* dam-calf contact [20 min before milking, until weaning] and a *Day-time* dam-calf contact [6-9 h/d until weaning]) to simulate the impact of their implementation in several French dairy farming systems. From a national technical-economic database (>1500 farms), we selected three "case-studies" representative of French dairy farming systems (extensive, intensive and organic) to simulate the economic results of the two suckling practices, and compare them to those of the conventional practice [calf separation at birth and artificial milk feeding until weaning].
Compared to the annual economic results of the conventional practice, the *Gross milk output* decreases in all simulations (-1 700 € to -6 636 €). However: 1/ in extensive system the *Gross operating profit* and the *Net farm income per associate* are lower for both CCC practices, with a lower impact for *Day-time* (-1 700 € and -850 €, respectively) than for *Short-time* practice (-11 300 € and -5 665 €, respectively); 2/ in intensive system, the *Gross operating profit* and the *Net farm income per associate* are higher for *Day-time* practice (+1 745 € and +661 €), due to a reduction in *Concentrate costs* (-1 782 €) and in *Employees and social costs* (-5 204 €). Conversely, they are much lower for *Short-time* practice (-20 905 € and -10 642 €), mainly due to increased *Employees and social costs* (+16 159 €); 3/ in organic system, the *Gross operating profit* and the *Net farm income per associate* are higher for *Day-time* practice (+1 243 € and +500 €), while they are much lower for *Short-time* one (-21 172 € and -10 772 €), mainly due to increased *Employees and social costs* (+15 335 €).
Although further studies are needed to refine these results, we may conclude that a day-time cow-calf contact for 12 weeks can be economically sustainable in intensive and organic farming systems.

Dairy farming systems, ethical production, cow-calf contact, suckling period, economic analysis

Introduction

The early separation of dairy calves, male and female, from their dams is a traditional practice, which apparently simplifies the work for farmers. Male calves are generally sold at 3 weeks of age for veal production and female calves, the future heifers, are artificially reared on the farm.
The promotion of more natural and ethical rearing systems for female calves, that allow cow-calf contact (CCC), needs to be tested in terms of farm profitability. For example, the reduction of production costs or workload, or the consumers' willingness to pay more for such production (Asheim et al., 2016) can be favorable points.
Previous studies have shown that implementing a CCC system may require changes in daily practices and long-term priorities of farms compared to conventional systems with early separation (Vaarst et al., 2020). Besides the technical changes, the new way of interacting and perceiving the animals can also be challenging for farmers; all these factors have to be taken into account during the transition to innovative practices (Ivemeyer et al., 2015; Vaarst et al., 2020). The growing interest in CCC rearing provides an opportunity to investigate the application of these practices in different types of farming systems (Vaarst et al., 2020).
The consequences of CCC systems on animal welfare, health and production have been widely studied and discussed in recent years, with many authors highlighting the positive aspects on animal welfare

(Knierim et al., 2020). However, the economic consequences of these systems have remained poorly studied.

The multitude of CCC rearing practices and farm factors that influence economic analysis make this type of study difficult to realize. Bickelhaupt and Verwer (2013) concluded that incomplete economic information on these alternative farming systems makes it difficult for farmers to consider a possible conversion. The lack of studies on this topic still makes it unclear what is the best approach to perform this type of economic analysis.

The aim of this study was to simulate, using a database, the impact of some female calves CCC rearing practices on the economic results of intensive, extensive and organic dairy farming systems, in France.

Materials and methods

Starting from the "Volame" experimentations on various CCC rearing practices set up at the INRAE (French National Institute for Agriculture, Food and Environment) experimental farm "Herbipole" in 2017, 2018 and 2019 (Nicolao et al. 2022, submitted; Nicolao et al., 2020), we selected two practices (a short-time dam-calf contact [called ***Short-time***] and a day-time dam-calf contact [called ***Day-time***]), with the aim of simulating the impact of their implementation in several French dairy farming systems, using "case-studies" from a national database called Diapason. For the *Short-time* practice, we used the data of the "Before" practice (2017) where dam-calf contact was allowed 20 min before morning and evening milkings during the first two weeks of lactation, and then 20 min before the morning milking, until weaning at 12 weeks. For the *Day-time* practice, we used the data of the "Dam" practice (2018 and 2019) where dam-calf contact was allowed during 6 to 9 h per day between the morning and the evening milkings, until weaning at 12 weeks. Each year, the suckling practices were compared to the conventional practice [called **Control**], where calves were separated from their dams immediately after birth and fed bulk milk with an automatic milk feeder until weaning.

Criteria selected and correction factors

In order to perform the economic simulation, we selected the criteria that, according to our knowledge, affect the farm's economic profit the most . Thus, the criteria selected were the amount of *milk sold,* the *milk composition*, the *calf growth* and the *workload*. For each criterion, we calculated the correction factors to be used in the simulation, based on data measured or estimated during the experiments. We took into account data on the dams of the female calves that will become the future heifers, and data on these female calves. We also investigated other criteria (such as cowshed design, cows' reproduction performance and animals' health disorders), but, as explained below, there was no evidence in the three experimentations that they affected the economic performance of a farm using CCC practices. Therefore, we did not consider the cowshed design, the cows' reproduction performance and the animals' health disorders in the economic analysis. As the aim of our study was not to evaluate the transition costs from a conventional rearing system to a CCC rearing system, we did not take into account the modification of cowshed layout. Indeed, we assumed that the barn surface needed to rear calves in a dedicated pen close to their dams is the same as rearing calves in a collective pen, elsewhere in the barn. The reproductive performance of the cows (percentage of cows pregnant, percentage of cows calving the following year, calving to first insemination interval, calving to fertile insemination interval, and calving to calving interval) recorded during the three trials showed no differences between the groups [data unpublished, in accordance with Krohn (2001)]. Similarly, health disorders for cows (mastitis, metritis, milk fever, lameness, etc.) and for calves (diarrhea, umbilical infection, dyspnea, etc.), recorded during the three trials, were few and not different between groups.

Milk sold correction factor. Starting from measurements performed in 2017, we estimated that the daily milk intake of calves fed almost ad libitum by their dams corresponded to 11% of their body weight (BW). We were therefore able to estimate the calves' milk intake until weaning in the *Short-time* and *Day-time* groups. From the lactation curves, we considered the milk yield at parlour of suckled cows until it reached the same level as the *Control* cows. Thus, in the *Short-time* group we considered milk yield up to week 20, while in the *Day-time* group up to week 12, and we assumed that the milk production after this period corresponds to the *Control* cows. By summing the total calves' milk intake and the total milk yield at parlour, we estimated the total milk yield of suckled cows. This allowed to calculate the "lost" milk, i.e. the difference between the total milk yield of the *Control* cows and the total milk yield of the suckled cows. Finally, the *milk sold* correction factor has been calculated as the sum of the calves' milk intake and the "lost" milk; it corresponds to the difference in milk that could be sold between the *Control* cows and the suckled cows (-985 kg/year for *Day-time* cows and -1 360 kg/year for *Short-time* cows, compared to *Control* cows; Table 2). We chose this method instead of a simple subtraction between total milk yield of *Control* cows and total milk yield of suckled cows because, in 2017 and 2018 there were the same number of suckled calves and dams (14), whereas in 2019, as male calves were removed from the experiment, there were only 9 calves for 14 cows. Therefore, as the female calves were also able to suckle the dams of the male calves, the milk losses of suckled cows in 2019 would not have been realistic.

Milk composition correction factors. They were calculated considering only the data of 2017 and 2018, when the number of calves was the same as the number of dams. They are determined as the difference between the average milk fat content and the average milk protein content of *Control* and suckled cows during the first 12 weeks of lactation, as milk composition returns to normal levels (i.e. those of *Control* cows) shortly after the weaning of all calves.
Based on this calculation, the fat content correction factors were -7.7 g/L for *Day-time* cows and +3.2 g/L for *Short-time* cows, compared to *Control* cows. The protein content correction factors were +1.4 g/L for *Day-time* cows and +2.4 g/L for *Short-time* cows, compared to *Control* cows.

Calf growth correction factor. It was determined as the average difference of body weight (BW) measured at weaning between *Control* calves and suckling calves: +12 kg for *Day-time contact* calves; +15 kg for *Short-time contact* calves. This allowed us to calculate the amount of concentrates needed for *Control* calves to reach the same weight as suckling calves in the weeks following weaning. According to INRA (2010), we assumed that for a heifer of about 250 kg, an energy intake of 5.0 UFL[2] (e.g. about 5 kg of concentrate feed) is required to increase its BW by 1 kg. Therefore, with a perspective of early mating, maternal feeding of calves allows a reduction in concentrate consumption after weaning from 60 kg (*Day-time contact*) to 75 kg (*Short-time contact*) per heifer.

Workload correction factor. The calculation of workload differences between CCC practices and the conventional one was not based on worktime measurements. The workload was estimated from personal experience in conducting the trials, then amended and validated by interviewing the staff who usually take care of animals at the experimental farm. During the first two weeks after calving, care is generally given individually to each calf, and then collectively after that date (Table 1).

[2] One UFL (Forage Unit for Lactation) is equivalent to 1 760 kcal of Net Energy for lactation

Table 1. Description of the difference in working time according to suckling practices from birth (Day1) to weaning (Week12), classified into workload per calf and per group of calves.

Workload		Task	*Control*	*Short-time contact*	*Day-time contact*
Per calf	Day1 & Day2	Colostrum[a] *(min/d)*	60	10	10
	Day3 to Day7	Calf monitoring[b] *(min/week)*	100	20	20
		Cow monitoring[c] *(min/week)*	-	210	210
	Week2	Cleaning[d] *(min/week)*	180	60	60
		Training calf[e] and moving animals[f] *(min/week)*	20	420	5
For a group up to 14 calves	Week3 to Week12	Feed control[g] *(min/week)*	160	-	-
		Moving animals[h] *(min/week)*	-	210	70

[a] *Control*: time to prepare and provide colostrum to the calf with a feeding bottle.
Short-time and *Day-time*: time to check if calf suckled properly its dam.
[b] *Control*: time to prepare and provide milk to the calf with a bucket.
Short-time and *Day-time*: time to check if calf suckled properly its dam.
[c] *Short-time* and *Day-time*: time to feed the dam individually.
[d] *Control*: time to clean hutch and pen of the calf.
Short-time and *Day-time*: time to clean individual calving pen of the dam-calf couple.
[e] *Control*: time to train the calf to suckle from automatic milk feeder.
[f] *Short-time*: time to move the calf to its dam for suckling and bring it back to calves' collective pen, twice a day.
[g] *Control*: time to check at automatic feeder if calves have been fed and to clean automatic feeder.
[h] *Short-time*: time to move calves to dams for suckling and bring them back to calves' collective pen, before morning milking.
Day-time: time to open and close separation gate to allow dams and calves to be in contact between milkings

Table 2. Summary of the correction factors calculated for each criterion selected for the economic simulation *(the data are in comparison to the data of the Control system).*

	Milk to be sold[a]	Milk fat content[b]	Milk protein content[b]	Calf weight at weaning[c]	Concentrate for heifers[d]	Workload[e]
Day-time contact	-985 kg	-7,7 g/L	+1,4 g/L	+12 kg	-60 kg	-13 h
Short-time contact	-1 360 kg	+3,2 g/L	+2,4 g/L	+15 kg	-75 kg	+41 h

[a] per year, for each cow that suckle her calf until weaning (12 weeks)
[b] on average, per L of milk milked at parlour (i.e. sold) for a cow that suckle her calf until weaning (12 weeks)
[c] for a female calf suckled by its dam for 12 weeks
[d] quantity of concentrate saved from weaning to first insemination for a female calf suckled by its dam
[e] per year for each dam-calf pair

Diapason database

The Diapason database was created by the « INOSYS Réseaux d'élevage » device to produce references on the functioning and sustainability of French farming systems. The references are produced annually by the advisors of the Chambres d'Agriculture, from farms classified according to the INOSYS typology, in partnership with the Institut de l'Elevage for the livestock sector. This database is structured around the identification of the means of production, the global functioning of the herd and the surfaces, the zootechnical performances of the herbivores and the economic results (Charroin et al., 2005). In the database, 14 regions are involved with 1509 farms monitored, from which different "case-studies" are developed. Each case-study groups real farms from a specific region and type of production; it is not an average of the data collected but the modelling of a coherent technical and economic functioning of one type of production in one region. The Diapason database and the simulation program were made available to INRAE to select case-studies that best represented the diversity of specialized dairy farming systems we wanted to investigate, and to perform economic simulations. For each case-study selected, we used a worksheet that allows us to apply correction factors on the parameters we identified, in order to perform a new economic simulation. For example, to calculate the milk sold in a CCC system, we multiplied the *milk sold correction factor* by the number of female calves in the system, and subtracted it from the total milk sold in the selected case-study. Farm structure and global functioning of the herd and the surfaces were kept constant. Once all the parameters were modified, the device automatically simulated the economic results of the CCC system, which can be directly compared with those of the initial case-study.

Case-studies selected

We chose to simulate the economic impacts of the CCC practices on three dairy farming systems (called "intensive", "extensive" and "organic"), widely representative of the diversity of specialised dairy systems that exist in France.

The intensive system chosen (Pays de la Loire 2019 – Système lait spécialisé, 50% de maïs dans la SFP, conventionnel[3]) is representative of the majority of French dairy farms, which are mainly located in the western plains of the country. It is a moderate-sized, often family-based, farming system using high-producing Holstein cows fed mainly with maize silage (Table 3).
The extensive system chosen (Auvergne 2019 – "BL17", système laitier spécialisé zone volcanique, système tout herbe, AOP Cantal et Bleu d'Auvergne[4]) is representative of a large proportion of French dairy farms located in mountain areas (>800 m a.s.l.). It is also a moderate-sized family farming system, based on the use of mixed breeds of cows, less precocious (e.g. Montbéliarde breed), fed mainly with grass (pasture, hay, silage). The milk produced is generally better valued than in lowland farms because it is often used to produce dairy specialties with quality labels (e.g. PDO cheeses).
The organic system chosen (Pays de la Loire 2019 – Système lait spécialisé, entre 5 et 15% de maïs dans la SFP, agriculture biologique[5]) is representative of French dairy farms located in the plains and complying with the European specifications for organic farming. It is a family system of moderate size, often using Holstein cows fed with a reduced amount of maize silage during winter and pasture during summer. The milk produced is well valued but the purchased concentrates are expensive. In addition, the organic specifications requires that calves "*shall be fed on maternal milk in preference to natural milk, for a minimum period of three months*"[6], which forces the farmer to allocate a large proportion of his milk to the rearing of female calves.

[3] Pays de la Loire 2019 – Specialized dairy system, 50% maize in the forage area, conventional farming
[4] Auvergne 2019 – "BL17", specialized dairy system in volcanic area, full grass system, milk for Cantal and Bleu d'Auvergne PDO cheeses
[5] Pays de la Loire 2019 – Specialized dairy system, 5 to 15% of maize in the forage area, organic farming
[6] The Commission Regulation (EC) No. 889/2008 of 5 September 2008, Chapter 2, Section 3, Article 20.1.

Table 3. Description of the main characteristics of the three case-studies selected from the Diapason database.

	Extensive	*Intensive*	*Organic*
Forage feeding	Grass *(100%)*	Maize (50%) & grass	Grass & Maize (5-15%)
Location in France	Auvergne	Pays de la Loire	Pays de la Loire
Workforce *(in AWU[a])*	2.0 associates	2.0 associates + 0.4 employees	2.0 associates + 0.3 employees
Dairy cows	57	78	79
Replacement heifers	14	27	25
Breed	Montbéliarde	Holstein	Holstein
Age at first calving	34-36 months	27 months	29 months
Milk production *(per cow/year)*	~5 600 L	~8 300 L	~5 700 L
Milk sold *(per year)*	300 000 L	630 000 L	430 000 L
Milk fat/protein contents *(g/L)*	40.0 / 32.5	42.3 / 33.8	41.5 / 31.8
Milk price *(€/1000 L)*	396	358	474

[a] Annual Work Unit

Economic data

The economic data used for this study are the original Diapason data for the year 2019. Some of these data are identical for the three case-studies, such as the amount of the SMIC (French minimum wage) but most of them (price of milk, of calves, of cull cows, of concentrates, aids, etc.) are specific to each case-study, depending on the type of production (organic or conventional; Holstein or Montbeliarde breed; etc.) and its location (plain or mountain). When simulating the different CCC practices, the most of these economic data do not vary within the same case-study (price of calves, of cull cows, of concentrate, aids, etc.), with the exception of the milk price. Indeed, the milk price can vary greatly depending on its composition in fat and protein contents, according to the specific scales of each dairy company and each type of production (organic or conventional). Within the framework of this study, we have elaborated a price grid established from the average of nine dairy factories distributed all over the French territory (Table 4).

Table 4. Grid of differential payment of milk to farmers, according to fat and protein contents *(average of nine dairy factories distributed all over the French territory)*.

Farming system	Milk fat content		Milk protein content	
Conventional	>38 g/L	+2.77 €/1 000L per g	>32 g/L	+6.28 €/1 000L per g
	<38 g/L	-2.77 €/1 000L per g	<32 g/L	-6.28 €/1 000L per g
Organic or PDO	>38 g/L	+4.86 €/1 000L per g	>32 g/L	+6.99 €/1 000L per g
	<38 g/L	-4.86 €/1 000L per g	<32 g/L	-6.99 €/1 000L per g

Economic results

The main economic outputs from Diapason are the *Gross operating profit* (€/year) which is an indicator of the profitability of the farm through its production system and the *Net farm income per associate* (€/year) which is an indicator of farmer's remuneration. The annual *Gross operating profit* is the difference between the annual *Total gross proceeds* and the annual *Total expenses*. The *Total gross proceeds* is the sum of *Gross milk output* and other products that are not impacted by CCC practices (e.g. animal sales, crops product or subsidies). The *Total expenses* is the sum of *Operating expenses* (as *Concentrate costs*, and other costs not affected by CCC practices [e.g. veterinary costs and fertilisers]) and *Structural expenses* (as *Employees and social costs*, and other costs not affected by CCC practices [e.g. land, buildings and equipment]). The *Net farm income per associate* is the difference between *Gross operating profit* and *Annuities* (e.g. capital repayment and financial costs, not impacted by CCC practices), divided by the number of associates.

Results

Extensive system

Compared to the Diapason case-study, the annual reduction in milk sold is higher in the *Short-time contact* system than in the *Day-time contact* system (-2.99% and -1.24%, respectively; Table 5). The *Milk price* is reduced in the *Day-time contact* system because it is penalized by the strong reduction of milk fat content of nursing dams (-7,7 g/L), while it increases in the *Short-time contact* system thanks to higher milk fat and protein contents (+3,2 and +2,4 g/L, respectively). In both CCC systems, there is a reduction in *Gross milk output* (-0.91% and -1.68%, respectively). In extensive systems the purchase of concentrate is not affected by the CCC practices because, with an average age at first calving of about 36 months, the heifers can usually reach the recommended weight at mating (about 27 months) without the use of concentrate, regardless of their weight at weaning. Considering that in the extensive system the workforce is composed of two associates and that this number cannot be reduced, in the *Day-time* system there is therefore a reduction in annual workload of about 182 hours for the two associates. As a result, in this system, *Gross operating profit* and *Net farm income per associate* are only impacted by the reduction in *Gross milk output*. In the *Short-time* system there is an increase in workload of +0.32 Annual Work Unit (AWU) which must be covered by an increase in the employed workforce. This increase of AWU in the *Short-time* system affects the *Employees and social costs* (+8 216 €) and consequently reduces the *Gross operating profit* and the *Net farm income per associate* (-11 330 € and -5 665 €, respectively).

In conclusion, in extensive systems the *Gross operating profit* and the *Net farm income per associate* are lower in both CCC systems, with a lower impact for the *Day-time* system than for the *Short-time* system.

Table 5. Technical and economic simulation of the impact of a *Day-time* and a *Short-time* dam-calf contact practice on extensive, intensive and organic dairy farming systems.

	Technical performance			Annual economic results					
	Milk sold (per year)	Concentrates purchased (per year)	Workforce (AWU)*	*Milk price* (/1000 L)	*Gross milk output* (€)	*Concentrate costs* (€)	*Employees and social costs* (€)	*Gross operating profit* (€/year)	*Net farm income per associate* (€/year)
Extensive system	***300 000 L***	***95 t***	***2.0***	***396 €***	***185 931***	***31 387***	***12 802***	***85 540***	***27 906***
Day-time contact	-3 708 L	-	*(-182 h/year)*	-0.80 €	-1 700	-	-	-1 700	-850
Short-time contact	-8 964 L	-	+0.32	+1.50 €	-3 115	-	+8 216	-11 330	-5 665
Intensive system	***630 000 L***	***147 t***	***2.4***	***358 €***	***225 578***	***49 477***	***26 145***	***89 619***	***22 155***
Day-time contact	-12 567 L	-1.59 t	-0.19	-1.20 €	-5 241	-1 782	-5 204	+1 745	+661
Short-time contact	-22 317 L	-1.99 t	+0.59	+2.20 €	-6 636	-1 890	+16 159	-20 905	-10 642
Organic system	***430 000 L***	***71 t***	***2.3***	***474 €***	***203 657***	***31 960***	***30 715***	***105 363***	***28 242***
Day-time contact	-6 625 L	-1.5 t	-0.18	-2.40 €	-4 158	-472	-4 929	+1 243	+500
Short-time contact	-16 000 L	-1.88 t	+0.56	+2.80 €	-6 427	-590	+15 335	-21 172	-10 772

* Annual Work Units. In each system two AWU, representing the two associates, are fixed; above that, employed workforce is involved.

Intensive system

The reduction in milk sold is higher in the *Short-time* system than in the *Day-time* system (-3.54% and -1.99%, respectively; Table 5). The *Milk price* decreases in the *Day-time* system because of the reduction of milk fat content, but it increases in the *Short-time* system thanks to higher milk fat and milk protein contents. Therefore, the combination of the decrease in the amount of milk sold and the change in its composition leads to a reduction in *Gross milk output* of about 6 000 € in each CCC system. In both CCC systems there is a reduction of the amount of concentrate purchased for heifers of about 1.8 t/year, which is higher in the *Short-time* system than in the *Day-time* system because the calves have a higher weight at weaning. As a result, in both scenarios there is a reduction in *Concentrate costs* of about 1 800 €/year. In the Diapason case-study of the intensive system, in addition to the two associates, 0.4 AWU come from employees who can provide an adjustment variable for workload variations related to the CCC practices. Therefore, in the *Day-time* system there is a reduction of about 0.2 AWU, while in the *Short-time* system there is an increase of about 0.6 AWU. This positively affects the economic result of the *Day-time* system with a reduction of the *Employees and social costs* (-5 204 €), which allows an increase of the *Net farm income per associate* of 661 €/year. Conversely, the increase of workforce negatively affects the *Short-time* system with an increase of the *Employees and social costs* (+16 159 €) which, combined with a reduction of the *Gross milk output*, leads to a very strong decrease of the *Net farm income per associate* (-10 642 €).
In conclusion, in intensive systems the *Gross operating profit* and the *Net farm income per associate* are higher if using *Day-time* contact practice because, despite the reduction of *Gross milk output*, there is a reduction in *Concentrate costs* and in *Employees and social costs*. While, the *Gross operating profit* and the *Net farm income per associate* are lower with *Short-time* contact practice, mainly due to increased *Employees and social costs*.

Organic system

The reduction in milk sold is higher in the *Short-time system* than in the *Day-time* system (-3.72% and -1.54%, respectively). The *Milk price* decreases in *Day-time* system because it is penalized by the reduction of milk fat content and it increases in the *Short-time* system thanks to higher milk fat and milk protein contents, as in the previous systems. Again, in both CCC systems, there is a reduction in *Gross milk output* (-2.04% and -3.16%, respectively). As in the intensive system, both CCC systems lead to a reduction of the amount of purchased concentrate for heifers and consequently a reduction in *Concentrate costs*. In the Diapason case-study of the organic system, in addition to the two associates, 0.3 AWU come from employees who can provide an adjustment variable for workload variations related to the CCC practices. Therefore, in the *Day-time* system there is a reduction of about 0.2 AWU, while in the Short-time system there is an increase of about 0.6 AWU. This positively affects the economic result of the *Day-time* system with a reduction of the *Employees and social costs* (-4 929 €), which allows an increase of the *Net farm income per associate* of 500 €/year. Conversely, the increase of workforce negatively affects the *Short-time* system with an increase of the *Employees and social costs* (+15 335 €) which, combined with a reduction of the *Gross milk output*, leads to a very strong decrease of the *Net farm income per associate* (-10 772 €).
In conclusion, as in the extensive scenarios, the *Gross operating profit* and *the Net farm income per associate* are higher in the *Day-time* system, while, they are lower in the *Short-time* system, mainly due to increased *Employees and social costs*.

Discussion

The economic simulation showed that regardless of the farming system, a *Short-time* dam-calf contact before milking has a negative economic impact on the farm's *Gross operating profit*. This is partly due to the higher milk loss, which despite a better milk composition (and therefore a better *Milk price*), cannot compensate for the reduction in *Gross milk output*. Nevertheless, the negative economic impact is essentially due to the high workload compared to a conventional rearing system or *Day-time* contact system. The increased workload is mainly due to moving animals, as once a day (and often twice due to the spacing of calvings) the farmer has to bring the calves to the cows, check that they suckle and bring them back to their collective pen. This type of restricted CCC system is already practiced in France in dairy farms with Salers cows. Indeed, cows of this breed can only be milked with their calf present. The level of workload required to move the calves twice a day is thus gradually reducing the economic interest of this traditional dairy breed. For this reason, dairy farms represent only 5% of the Salers farms (Agabriel et al., 2014).

The comparison between the three farming systems showed that the extensive system was the only one where the *Gross operating profit* decreased with both CCC practices. As expected, the higher weight at weaning of female calves does not affect the heifers' weight at first insemination, and consequently the purchase of concentrates by the farm. Moreover, we were not able to consider economically the reduction of workload with the *Day-time* practice because the structure of the Diapason case-study is based on a farm with two full-time associates. Thus, even if there is a reduction in workload for the associates, this reduction does not affect the economic result; we can suppose that there was an improvement in working conditions and that the time saved could be re-thought and reallocated to other tasks, on or off the farm.

It should be noticed that the reduction of 182 h of workload per year for 14 female calves that we estimated for the *Day-time* practice in an extensive farming system is quite comparable to the 10 h reduction in workload per calf reared with its dam assumed by Asheim et al. (2016) in their economic analysis based on surveys. Whereas in our study the workload was not measured but estimated, and we considered only one farm where the different CCC systems were tested.

Vaarst et al. (2020) also reported in their study that most interviewed farmers emphasized the need to reorganize workload rather than looking for time and work savings. Other studies on farmers' perceptions of CCC systems reported that reduced workload is one of the main advantages of these practices. Michaud et al. (2018) showed that the main motivation of farmers to implement CCC systems was the reduction of working time (46% of interviewed farmers), before the reduction of livestock costs (31% of interviewed farmers). Similarly, a recent survey on the spreading of CCC practices in Europe showed that on 103 farms in 6 EU countries, 43% stated a gain in working time as a driver of the implementation of this practice, in particular in France where 20 out of 25 farms practicing dam-calf rearing reported a saving in working time (Eriksson et al., 2022, under submission).

Our results showed that a *Day-time* contact system for 12 weeks seems to be advantageous for the farmer both in intensive and organic farming systems, mainly due to the reduction of workload, but also to the reduction of the concentrates costs. Knierim et al. (2020) analyzed a case-study with permanent CCC system for 13 weeks and concluded that, under certain conditions, rearing cows and calves with full contact could be economically advantageous.

Other positive aspects associated with a CCC practice could be a higher price paid for male calves due to the higher weight gain reach after the suckling period, but in our study we did not take into account male calves sold at 3 weeks old. Moreover, the application of future labelling initiatives on the price paid for calves reared with their dams could encourage this practice, even if it seems that higher prices are already applied for the sale of calves reared in this way (Bickelhaupt & Verwer, 2013).

Our study shows that the practice of *Short-time* contact seems to be a real economic problem because of the increase in workload associated. On the other hand, we wonder if the reduction in workload associated with *Day-time* contact (-13 hours per calf reared by its mother) will have a real economic impact on family farms for which this time-saving does not necessarily justify a reduction in employed workforce. However, considering only the sale of milk, the adoption of a *Day-time* contact system for 12 weeks would lead to an annual reduction in milk income that could be compensated by a milk price increase of about 8 €/1000 L, i.e. less than 1 Eurocent per liter.
Thus, CCC systems may become more profitable if consumers, due to animal welfare concerns, are willing to pay more for milk and veal from such production systems. As suggested by Asheim et al. (2016), some type of regulation could also be implemented to claim these practices, if desired by the public.

Conclusion

We may conclude that a *Day-time* contact system for 12 weeks can be economically sustainable both in intensive and in organic farming systems, since, despite the loss of milk due to suckling, there is also a reduction and an improvement of working conditions, associated with a better growth of calves. Moreover, the reduction in the milk price due to the modification of its fat and protein content is marginal, so this practice would be economically profitable if we assume the application of a label for the milk produced by this kind of system. Actually, it is difficult to predict economic changes on a theoretical basis in systems involving CCC, because there are a large variety of rearing systems with as many number of interaction factors, even if there are common factors among the different systems such as the reduction in the amount of saleable milk or the increased growth of calves. We chose to apply a modeling method on case-studies, but if CCC practices will widely develop we can consider studying their economic consequences from real data surveys. Finally, further investigations could be implemented on the economic impact of CCC systems by performing sensitivity analysis on milk price and on workforce, as well as to assess how work organization is affected by such practices.

Acknowledgements

The authors thank the French « INOSYS Réseaux d'élevage » for the provision of the Diapason database and its simulation tool.

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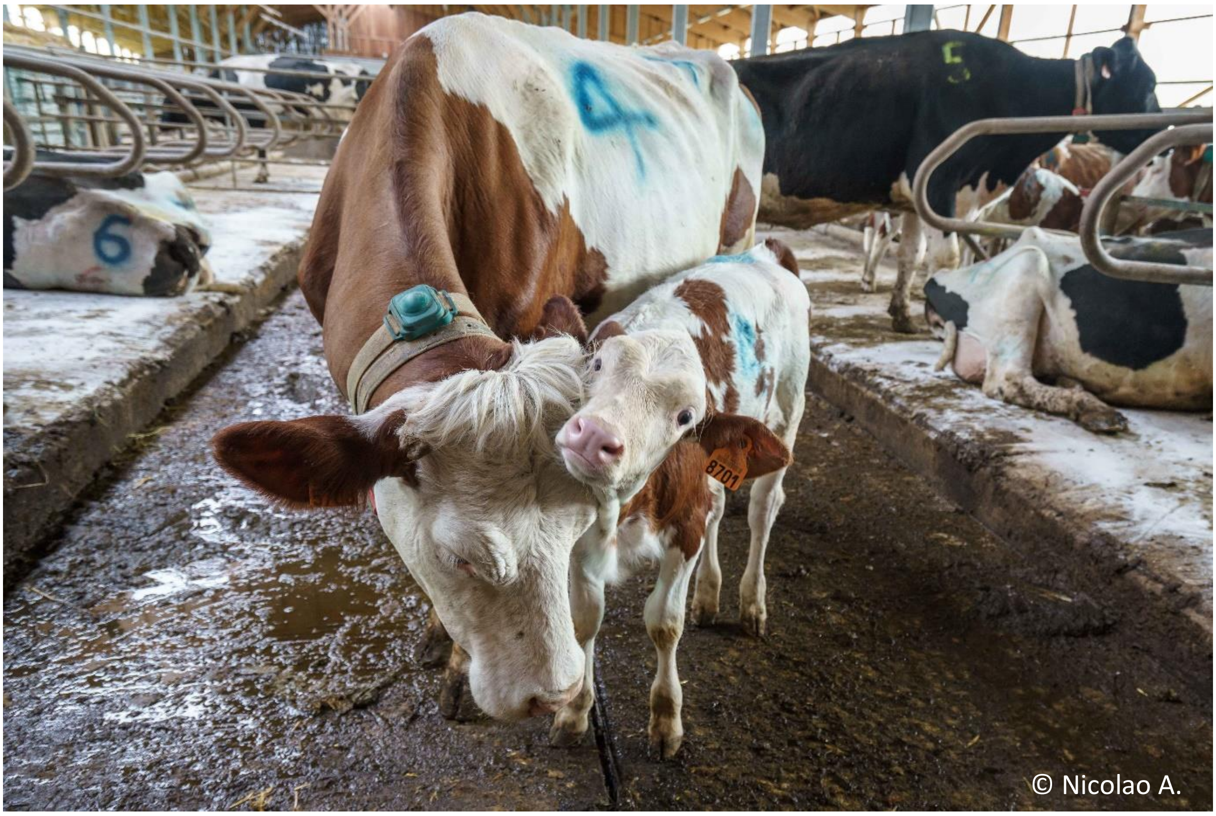
CHAPTER 6

General discussion



In a context of a growing societal concern on livestock farming conditions in general and animal welfare issues in particular, the aim of this doctoral thesis was to experimentally investigate different dairy calf rearing systems allowing cow-calf contacts (CCC).

Initiated from the experience of pioneer farmers who implemented innovative CCC practices on-farm, the first objective was to quantify the consequences of these practices on animal performance and stress at weaning. We compared experimentally four different CCC practices to artificial rearing practices (*Control*) in order to find out the practice corresponding to the best trade-off between animal welfare, animal performance and economic impact. In Trial 1, we tested two short-time CCC practices, where calves had access to their dams for a short period every day until weaning (20 min '*Before*' and 2.5 h '*After*' morning milking) (Chapter 2). In Trial 2, we tested a day-time CCC, where calves had access to their dam between the two daily milkings ('*Dam*', 9 h/day) (Chapter 2). In Trial 3, we tested two day-time CCC ; in the first one the calves had a day-time access to dams until weaning (*Dam*, 6 h per day) while in the other one the calves had a day-time access to dams until 4 weeks of age before being separated from their dam and reared as calves of the Control group until weaning (*Mixed*) (Chapter 3). Moreover, in Chapter 3, we furthered the study of the consequences of suckling on cows' blood metabolites, on the passive transfer of immunity from cows to calves and antibodies in suckling cows' milk, and on cortisol in hair of calves. The second objective was to investigate if the early-life dam-calf contact and grazing experience influence post-weaning behavior and herbage selection of dairy calves in the short term after the turn out to pasture. In Trial 3, we therefore investigated the effect of three different early-life experiences on the calves' grazing and social behavior after weaning (Chapter 4). Finally, the third objective was to simulate the economic impact of two suckling practices (*Short-time contact* and *Day-time contact*) on three different farming systems in France (Chapter 5).

This is one of the few studies available in literature that gives an overview of the consequences of CCC systems on animals' performance, animals' health, calves' grazing behavior, weaning distress and finally on the economic viability of these calf rearing systems.

The general discussion is structured in three sections. In the first section, I will examine the consequences of CCC on cows and calves behavior during the pre- and post-weaning period, starting from the results presented in this thesis and adding some new results never presented before. In the second section, I will examine the consequences of different CCC on animal performances across the different trials. Finally, in the third section, I will provide an overview of the viability of implementing CCC practices in different dairy systems.

6.1. Animals, behavior and welfare

6.1.1. *Which is the best CCC system in terms of cow-calf bond and calves behavior?*

Societal concerns about the traditional practice of separating the newborn dairy calf from its dam have prompted several research questions about how to manage systems that allow CCC and their consequences on animal welfare (De Oliveira et al., 2020). CCC systems provide increased opportunities for the expression of natural behaviors, such as nursing and bonding, and, for calves, learning social behaviors from adults of their own species. Improving these aspects can promote the sustainability of the future dairy sector in terms of consumer confidence (von Keyserlingk et al., 2013).

As introduced in Chapter 1, different types of CCC practices exists, but the main distinction is between dam-calf contact rearing, when physical contact is allowed between the dam and its own calf, and foster-cow rearing, when a cow nurses one or more foster calves (Sirovnik et al., 2020).

It might be expected that the best compromise between animal welfare and economic impact could be the implementation of a foster-cow rearing as this practice allows exploiting cows with high somatic cell count or subclinical mastitis that would impair milk price if sold (Loberg and Lidfors, 2001).

However, although there are few studies on this, there is a risk of mastitis-associated pathogen transfer between cow and calf during suckling (Köllmann et al., 2021). Advantages of foster-cow rearing also include the fact that several calves can be kept together and suckle one cow, that calves can live in groups, have contact with adult cows, and have natural suckling behavior (Loberg & Lidfors, 2001). However there are also many disadvantages. The main difficulty is ensuring that the foster cow accepts alien calves, and that cow is able to create a bond with them. Although most foster cows accept alien calves (Loberg & Lidfors, 2001), this may be time consuming for the farmer and fostered calves often receive less affiliative behaviors from the foster cow compared to the cow's own calf as the foster cow may often show preference for one specific calf (Johnsen et al., 2016). A calf is accepted or tolerated by a foster cow when it suckles in the reverse parallel position (Le Neindre and Garel, 1979) (Figure 6.1a). Moreover, the weight gains of calves suckling foster cows could be highly variable, especially when the cow has a low milk production (Johnsen et al., 2016). Nevertheless, it also depends on the relationship with the cow: when two calves were allowed to suckle a cow, including one's own calf, it has been found that the poorer was the relationship between cow and alien calf, the greater was the difference between the weight gain of the two calves (Le Neindre, 1989). Finally, foster cow and calves show behavioral reactions to separation indicative of considerable stress (Loberg et al., 2007; Loberg et al., 2008) and therefore in both foster- and dam-calf rearing systems, it is necessary to implement alternative measures to reduce stress at weaning or at separation.
Allowing cows to rear their own calf provides expression of behaviors associated with maternal care, creation of mutual bond, protection and provision of nourishment and finally breaking this bond at weaning (von Keyserlingk and Weary, 2007).

In Trial 1, suckling calves spent only three days after birth in individual calving pens with their dam or even in a collective calving pen (depending on pen avaiability). Successively, Before-calves had access to their dam in a specific collective pen facing the milking parlour for 20 min before the morning milking and After-calves for 2.5 h after the morning milking (see Chapter 2).
Since the time for suckling was restricted and the collective park was not very large (90 m^2 for 14 cow-calf couples), the moment of cows and calves reunion was characterized by a great confusion, marked mostly by the eagerness of the calves to gain access to the first available cow to suckle. Even if we did not make behavioral observation on dam-calf bond, we could assume that the duration of dam-calf contact was not enough to allow the formation of a strong bond between dam and calf or to allow cows to express maternal behavior. Moreover, results on calf growth performance in the After group, showed that restricted contact immediately after milking was not sufficient to cover the calves' nutritional needs. While results on vocalizations at weaning showed that on average 22% of Before-cows vocalized frequently during the two days following the weaning compared to 89% of Dam-cows in Trial 2 and 68% of Dam-cows in Trial 3. This weak reaction of cows to calves' weaning may confirm a lack of bonding between the dam and its calf.

In Trial 2, behavioral observations were made before weaning on dam-calf bond and on calves' behavior during the day, but we did not reported the results in Chapter 2. The aim was to investigate the effects of dam's presence on the behavior of calves and on the effects of breed on dam-calf bond. Dam-calves spent the first five days after birth alone with their dam in an individual calving pen before being housed in a collective pen next to the cowshed until weaning (see Chapter 2).
From the beginning of March to the start of the grazing period (beginning of May), once a week, in addition to the observations on the *acceptance by cows of calves other than their own* (i.e. all the calves' successful and refused suckling attempts from their dam or from other cows), presented in Chapter 2, the *latency time* of calves to reach its own dam and the *suckling position* (Figure 6.1) were recorded during the "Morning reunion". Moreover, all the calves were observed in the morning and in

the afternoon by focus observation of 2x10 minutes per calf to record general and social activities ("Daily activities"). These observations allowed to characterize the behavior of calves reared with their dams (Dam-calves) and without their dams (Control-calves) during the day.
The activities recorded were classified in *individual activities* (i.e. ingestion solid feed, suckling, rumination, exploration, self-grooming, locomotor play etc.), *social interaction with other calves* (positive and negative interactions) and *social interactions of Dam-calves with dam and other cows* (sniffing, licking, suckling etc.).

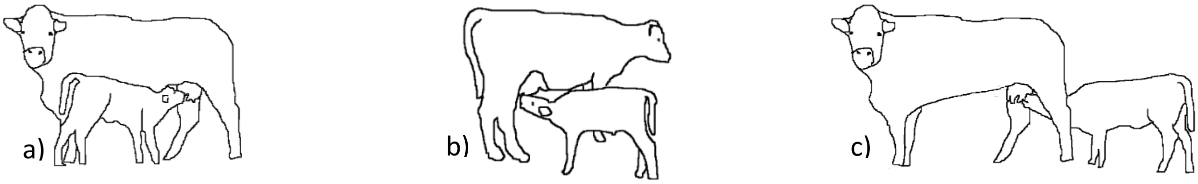


Figure 6.1. Sulking positions; a) Reverse parallel suckling position; b) Anti-parallel suckling position; c) Behind suckling position.

6.1.2. Effect of dam-calf contact on calves' behavior before weaning

Results of *Daily activities* observations showed that the cows' presence affected the general activities of calves (Dam- and Control-calves) only on the time spent suckling during the day. Dam-calves were more often observed suckling their dam than Control-calves were seen at the milk feeder (17% vs 7% of total observation time respectively, P = 0.001), as previously reported by Fröberg and Lidfors (2009) and Veissier et al. (2013).
This could be explained as Dam-calves had restricted time to suckle during the day, while Control-calves had access to the milk dispenser 24 h/day. However, another explanation could be that cow' presence stimulates the calf's motivation to suckle. Indeed, Roth et al. (2009) showed that milk intake via artificial teat does not fully satisfy the calves' motivation to perform suckling behavior, therefore Dam-calves may be motivated to suckle their dam not only because of hunger but also because of the need to satisfy the natural behavior to suckle.
Nevertheless, the presence of cows influenced calves' social interactions. We found that, in total, calves reared with their dam had more positive interactions than artificially reared calves (P = 0.0001; Figure 6.2 and Figure 6.3), as reported by Flower and Weary (2001). Le Neindre and Sourd (1984) also found that dam-reared calves showed higher levels of social activity than calves separated soon after birth. In our study, the most positive interactions were with adult cows, whereas positive interactions between calves were lower in Dam- than in Control-calves (P = 0.001; Figure 6.2). As reported by Krohn et al. (1999), maternal presence is important for calves' social learning and can reduce fearfulness of others. Contact with dam and other cows during milk feeding period could give to calves more opportunities for social experiences (Wagner et al., 2013). Adult cows impose social models, rules and herd's hierarchy from the early age and this may have positive effects on calves' social behavior when they will become adults (Bøe & Færevik, 2003). Furthermore, the maintenance of close proximity between dam and young provides opportunities for social transmission of information, such as information about feed sources or awareness of dangers (Newberry and Swanson, 2008).
Cows' presence also affected negative interactions among calves: we found no negative interactions (i.e. pushing, kicking, head-butting) between Dam-calves, whereas Control-calves showed more frequently negative interactions between each other (34% of total observation time ; P = <.0001). This is in opposition to Le Neindre and Sourd (1984) who found that heifers reared with foster cows dominated more than heifers reared without cow contact.

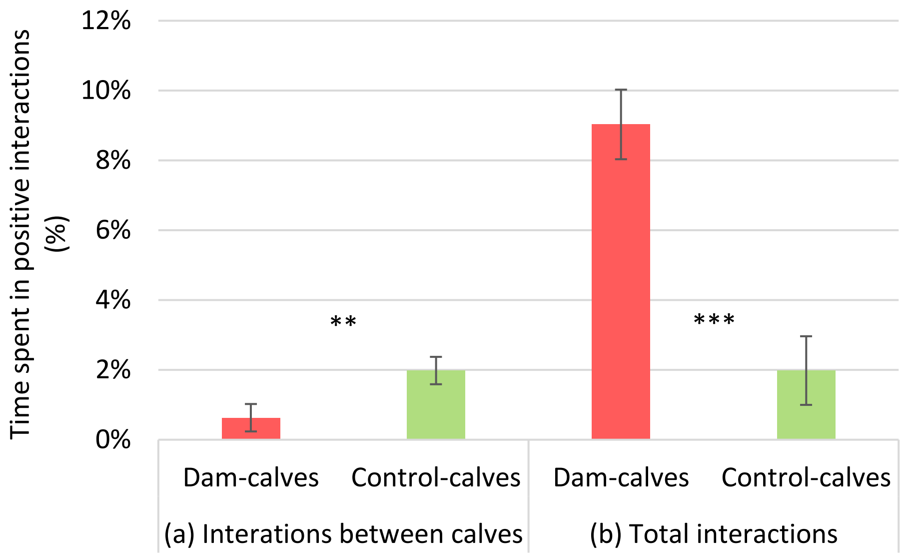


Figure 6.2. Time (%) spent performing positive interactions with other calves (a) or with all individuals (b) according to the group. LMM, *** P < 0.001; ** P < 0.01.

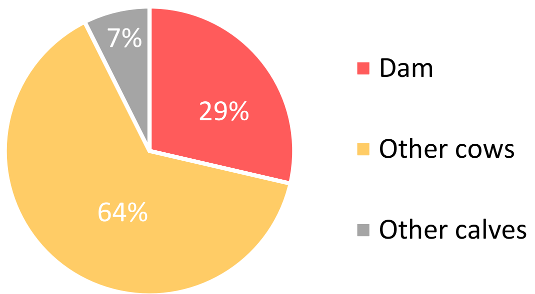


Figure 6.3. Time (%) spent by Dam-calves performing positive interactions with their dam, other cows and other calves.

In Trial 3, we also investigated the effect of dam-calf contact in comparison to conventional rearing without contact to the dam on chronic stress in calves until weaning, by analysing cortisol hair concentration in calves before and after weaning (Chapter 3). We found that calves reared with their dam until weaning (Dam-calves) had a significantly lower hair cortisol content before weaning than calves separated at birth or after 4 weeks (Control- and Mixed-calves). Considering that Dam-calves had presumably a higher level of activity as they were grazing with their Dam during the pre-weaning period than Mixed- and Control-calves that stayed inside the barn until weaning, we concluded that the level of chronic stress of calves reared with their Dam until weaning was lower. It may counterbalance partly their higher stress level found at weaning. Our findings on chronic stress revealed by hair cortisol concentration are in line with Lupoli et al. (2001) who showed that higher oxytocin levels in calves' plasma found in response to suckling were followed by a decrease in cortisol, 30 min after onset of suckling, which may reflect an anti-stress situation induced by the high levels of oxytocin.

6.1.3. Effect of breed on dam-calf bond

As reported in Chapter 2, we found that Montbéliarde (Mo) cows were more reluctant to accept another calf from their own, while Holstein (Ho) cows rarely made this distinction. Going deeper into the results on "Morning reunion" observations, we found that at morning, Mo calves spent more time suckling other cows than their own dam, compared with Ho calves (Figure 6.4). Johnsen et al. (2015b) observed that calves suckled another cow at least once before reaching its own dam, and they concluded that calves' strong motivation to access milk when first reunited led them to approach the first available cow to suckle before moving to their dam.

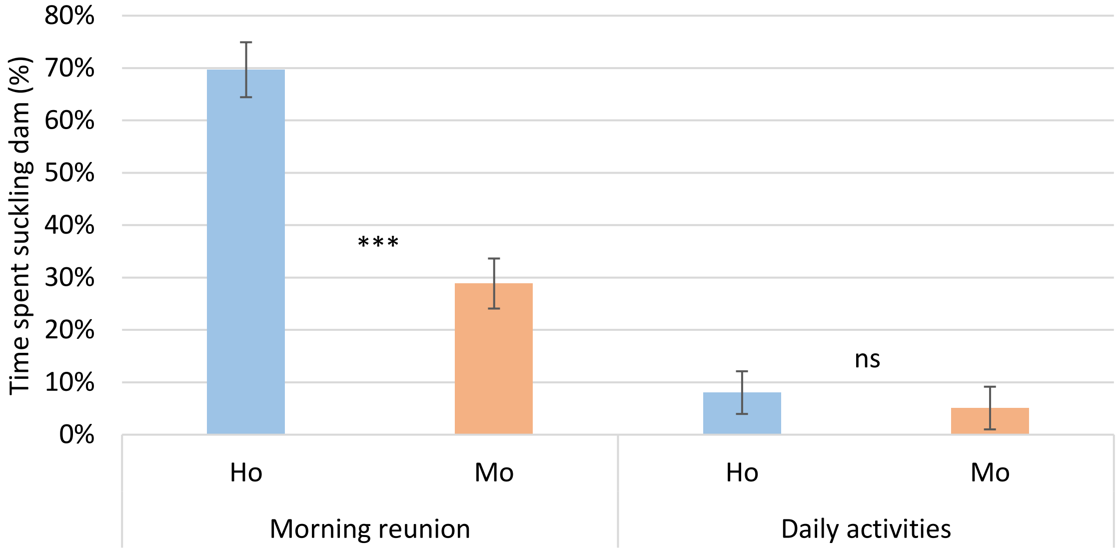


Figure 6.4. Time (%) spent by Dam-calves suckling their dam at morning reunion and during the daily activities, according by breeds. LMM, *** $P < 0.001$; ns $P \geq 0.10$.

Le Neindre (1989) stated that it is not only the milk availability but also the teats morphology and the easy milk ejection that influence the choice of a hungry calf seeking to milk. On average, Mo calves recorded a higher number of *successful suckling attempts* than Ho calves ($P = 0.005$), and specifically from other cows than their own ($P = 0.006$) (Table 6.1). This could be explained as the calves did not suckle their dam, so they had to continually change the cow to suckle. Indeed, when a calf suckles another cow, it usually takes the behind or anti-parallel position to avoid that cow recognize it by smelling or licking, and also, it normally suckles simultaneously with cow's own calf as strategy to reduce the probability of rejection or aggression from the cow (Fröberg and Lidfors, 2009) (Figure 6.5). On the contrary, when a calf suckles its own dam, it tends to suckle continually, with parallel position and for longer time because it is not rejected by the cow (Le Neindre and Garel, 1979). We found that at the morning reunion, Ho calves spent 70% of their time suckling their dam compared to Mo calves which spent 30% (Figure 6.4). This still may suggest that Mo cows tended to be more selective towards their calf than Ho cows. Le Neindre (1989) compared the maternal behavior of Friesians cows to Salers cows and found that Friesians cows were less selective towards alien calves. Friesians were suckled more by alien calves than were Salers, as Friesian cows were able to rear their own calves, but they seemed unable to forbid alien calves to suckle. They concluded that low calf selectivity was related to easy milking, as the milking ability of Friesians is higher than that of Salers.
In our study, however, the two breeds have a similar milking ability, so we assume that the Ho were less selective because of their easier milking. We did not find any breed effect on the *latency time* to reunite with own dam: all cows and calves normally reunited soon (about 4 minutes, on average; Table 6.1) after the gate opening, indicating mutual recognition and motivation to reunite. Moreover, the creation of the dam-calf bond occurred in all cows, regardless of breed, as we did not record any refused suckling attempts from dams towards their own calves.
We can conclude that both Ho and Mo cows had a good maternal behavior towards their calves. As reported by Le Neindre (1989), the method of rearing the dam can affect the bond to her calf: rearing cows with their calf in isolation around calving improve attachment. Keeping cow and calf separate from the herd after birth, as is the case in Trial 2, might facilitate the attachment between dam and

calf (Fröberg and Lidfors, 2009). Finally, it seems that free suckling during the day allows the development of a strong dam-calf bond, as the most calves suckled mainly their dam, in reverse parallel position and dams often licked their calves and were in close proximity to them.

Table 6.1. Cow-calf interactions during the "Morning reunion" observations.

		Ho (n=7)	Mo (n=7)	SEM	Breed
Morning reunion (1h)	Latency time (s)	271	232	62.6	*ns*
	Nb of refused suckling attempts from Dam	0	0		*ns*
	Nb of refused suckling attempts from Other cows	1,13	0,80	0.22	*ns*
	Nb of total successful suckling attempts	4,60	6,74	0.64	**
	Nb of successful suckling attempts from Dam	2,11	2,44	0.37	*ns*
	Nb of successful suckling attempts from Other cows	2,65	4,48	0.46	**

** P<0.01; ns P≥0.10

Figure 6.5. Alien calves suckled at the same time as cow's own calf to reduce the risk of rejection or aggression from the cow (INRAE 'Herbipole' experimental farm).

6.1.4. Effect of dam-calf contact on calves' behavior after weaning

Following this study on the effect of dam-calf contact on calves' behavior before weaning, in Trial 3 we investigated the effect of early dam-calf contact and grazing experience on calves' social behavior after weaning (Chapter 4).

Dam-calves were reared and grazed with their dams until weaning (Figure 6.6). Mixed-calves were separated from their dams after 4 weeks, they experienced dam-calf contact, but not grazing. Control-calves had never experienced either dam-calf contact (separated at birth) or grazing. Individual daily activities and behavior were observed by scan sampling at 5-min intervals on the day the calves encountered the pasture for the first time (Day 0), the next three days (Day 1, Day 2, Day 3) and one week later (Day 7).

As in Trial 2, calves reared with their dams showed fewer positive interactions with other calves, whereas calves separated at birth had more positive interactions with their companions (particularly licking) (see Chapter 4). Similar results were found by von Walter et al (2021) who found that goat kids permanently separated from their dams developed strong bonds with their flock mates and allowed them to adapt more easily to living in groups with other goat kids at the age of 2 months than goat kids separated daytime, who showed a stronger stress response at this age. Pinheiro Machado et al. (2020) found that licking behavior between grazing dairy cows was not a random choice but showed a companion's preference for socio-positive interactions. Moreover, they observed that licking was more persistent in long-established social groups. This could suggest that Dam- and Mixed-calves may have created bonds rather with dams than with other calves, compared to Control-calves, and this is consistent with the results of calves' behavioral observations on the before weaning period, in Trial 2. We thus expected that calves reared with their dams would be more sociable or have more dominant behaviors than artificially reared ones, but we found no differences in negative interactions between groups at pasture.

Finally, we found that early dam-calf contact and early grazing experience influenced calves' foraging skills in the very short term after weaning, especially on the first grazing day. The dam presence during the first grazing affected the calves' botanical selection throughout pasture utilization and their ability to reject toxic plants (as Rumex) showed that young calves could already exhibit post-weaning grazing behavior similar to that of adult cows when put on pasture early with their dam.

Moreover, on the post-weaning period, the difference found in hair cortisol during the pre-weaning period between groups tended to persist. Consistent with these results, in behavioral observations post-weaning, we found that Mixed-calves spent less time lying than the calves in the other groups and hypothesized that standing indicated a stress condition of the calves, especially under chronic stress (Wilcox et al., 2013).

Figure 6.6. Dam-calf pair grazing in the pre-weaning period, at INRAE 'Herbipole' experimental farm (Marcenat, Fr).

We can conclude that isolating the cow with her calf during the first days after calving could improve the dam-calf bond. Both Ho and Mo cows had a strong maternal behavior towards their calf, but Ho cows had more difficulty rejecting alien calves than Mo cows, and this may be due to their teat morphology and the easier milk ejection compared to Mo cows. A restricted dam-calf contact during the day did not affect the calves' general activities, but the dam's presence stimulated the calves' motivation to suckle more than the presence of an automatic milk feeder. The dam's presence, on the other hand, had an impact on the social behavior of calves in both the pre-weaning and post-weaning periods, particularly in the relationship between calves: if given the opportunity to choose, the calf prefers to interact mainly with adult individuals. Early social experience with the dam and other cows also had little impact on calves' grazing behavior, since in the short term after weaning, the calves grazed similarly to adult cows. Finally, rearing calves with their dam until weaning reduced the calves' chronic stress level at least before weaning. Therefore, we can suggest that the best CCC in terms of dam-calf bond and calves behavior is a day-time CCC until weaning.

6.1.5. Dam-calf contact and animals' weaning distress

As the experiment of After-calves was stopped at about 8 weeks of age (see Chapter 2) we do not have any results related to weaning distress for this group. However, in all 3 trials we calculated the daily percentage of animals that vocalized (frequently or from time to time) around weaning (the day before weaning [Day 0], the day of weaning [Day 1], and Day 2, Day 4 and Day 7 after weaning).

Table 6.2. Synthesis of the main results related to cow and calf distress at weaning in the three trials. In each trial, experimental groups were compared to a control group of animals. For each group, the day until a third of animals still vocalized and the maximum percentage of animals vocalizing during the week after weaning are reported.

		Trial 1	*Trial 2*	*Trial 3*			*3 Years*
		Before (20min/d, **weaning**)	**Dam** (9h/d, **weaning**)	**Dam** (6h/d, **weaning**)	**Mixed** (6h/d, **4wk**)		**Control**
					At separation (4wk)	At weaning	At weaning
COWS	**Day** until 1/3 of animals still vocalized	Day 2	Day 4	Day 2	Day 2	-	-
	Max % of animals vocalizing during the week after weaning	82%	100%	78%	57%	-	-
CALVES	**Day** until 1/3 of animals still vocalized	Day 4	Day 7	Day 4	Day 2	Day4	Day4
	Max % of animals vocalizing during the week after weaning	92%	100%	100%	94%	92%	89%

One of the critical points of dairy CCC is the later separation from the dam or weaning at the same time as the dam separation, because it is considered more stressful for both cow and calf than early separation (Flower & Weary, 2001). However, as reported in Table 6.2, we found that separation and weaning were very stressful events for calves because, whatever the group, almost all calves vocalized during the week after separation/weaning. Weaning has a psychological component due to the associated changes in environment: separation from the dam for calves suckling their dam, changes in accommodation (for all calves in our trials), and changes in feeding routines (Jasper et al., 2008; Veissier et al., 1989b; Weary et al., 2008).

The hypothesis that reducing the CCC period (4 weeks *vs.* until weaning) may reduce the distress at separation was not supported by our results. In the Mixed group we found that almost all the calves vocalized after both separation from the dam and weaning. The main difference was that at separation most of the Mixed-calves vocalized until Day 2 while at weaning until Day 4. This could be explained as at 4 weeks, calves still had access to milk through the automatic milk feeder, while at weaning they did not. As reported by Johnsen et al. (2018), vocalizations can be at least in part due to hunger since calves vocalize less when they have access to milk after the separation from their dams.

On average, we found that in the other groups most of the calves vocalized until Day 4 after weaning. Only Dam-calves in Trial 2 vocalized until Day 7, but we have no interpretation to explain this difference.

Separation seemed less stressful for Mixed-cows compared to Dam-cows, as calves were removed after a few weeks. Similarly, weaning of Before-cows was less stressful than weaning of Dam-cows, and this suggests that a short-time CCC may not allow for dam-calf bonding. However, on average, most of the cows vocalized until Day 2 after separation/weaning.

We can conclude that weaning is a stressful event for all calves, both those reared with their dams and those separated at birth. Reducing the dam-calf contact to a few weeks seems better for cows' distress, but it induces two periods of stress for calves (separation then weaning) instead of one (simultaneous separation and weaning). These pronounced behavioral responses showed an obvious welfare concern of CCC systems but also of conventional systems for calves.
However, the balance of CCC systems' benefits, in terms of animal welfare and expression of natural behaviors, is better for both calves and cows if systems that reduce stress at weaning are implemented, such as two-step weaning systems.

6.2. Animal performances

6.2.1 Consequences of cow-calf contact on cows' milk yield and composition

Our experiments allowed us to get an overview on the consequences of different CCC practices on calf and cow performances. Since the experiment was stopped for the After-group at about 8 weeks of age (see Chapter 2), we did not consider this practice in the general discussion.

Table 6.3. Consequences of the suckling practices on cows and calves performances during the 16 first weeks of lactation (Trial 1, 2 and 3). In each trial, experimental groups were compared to a Control group of animals. Differences between experimental and control groups reported in bold correspond to significant differences between experimental and control groups (P<0.05).

		Trial 1	*Trial 2*	*Trial 3*	
		Before (20min/d, **weaning**)	**Dam** (9h/d,**weaning**)	**Dam** (6h/d,**weaning**)	**Mixed** (6h/d, **4wk**)
COWS PERFORMANCE *Milk yield and composition at parlour during 16 weeks after calving*	**Milk yield** (kg/d – [%])	**- 10.6** [-45%]	**- 8.3** [-31%]	**- 7.8** [-30%]	**- 5.4** [-21%]
	Milk fat content (g/kg)	**+ 3.2**	**- 5.8**	**- 4.0**	- 0.8
	Milk protein content (g/kg)	**+ 3.0**	**+ 1.3**	**+ 1.0**	**+ 1.6**
CALVES PERFORMANCE	**Growth rate until weaning** (g/d)	**+ 171**	+ 7	**+ 251**	- 34

As shown in Table 6.3, which summarizes the main results of the three trials, milk yield at parlour was 31 % (-8.3 kg/d) and 30 % (-7.8 kg/d) lower in Dam-cows compared to Control-cows, in Trial 2 and 3 respectively. Interestingly milk yield at parlour was equivalent whatever the number of calves kept with dams (all calves in Trial 2 and female calves only [65%] in Trial 3). Milk losses at parlour were slightly lower when calves are separated from their dam at 4 weeks of age (Mixed-cows, -21%) but in this case, tank milk must be provided to calves after separation and until weaning.

One of the innovative aspects of this thesis was to be able to calculate the "lost" milk in each trial. Starting from measurements of milk ingested by calves, performed in Trial 1, we estimated that the daily milk intake of calves fed almost ad libitum by their dams corresponded to 11% of their body weight (BW). We were therefore able to calculate the calves' milk intake until weaning in Before group (Trial 1) and Dam groups (Trials 2 and 3), and the milk intake until 4 weeks in Mixed group (Trial 3). By summing the total calves' milk intake and the total milk yield at parlour, we estimated the total milk yield of suckled cows. This allowed to calculate the "lost" milk, i.e. the difference between the total milk yield of the Control cows and the total milk yield of the suckled cows (Figure 6.7).

Until the weaning of calves, in the three trials the reduction of sellable milk of suckled cows exceeded the amount of milk suckled by calves, and therefore total milk production was still lower than that of Control-cows by 22% in Before-cows (Week 13, Trial 1), by 13% and 12% in Dam-cows (Week 11, Trails 2 and 3, respectively). This difference is even more pronounced when calves suckled only during the first 4 weeks of lactation. Until Week 4, the total milk production of Mixed-cows was lower than that of Control-cows by 30% and, until Week 11, it was still lower by 18%.

This allowed us to find out that, contrary to what is often reported in the literature (Meagher et al., 2019), the loss of saleable milk does not only correspond to the milk drunk by the calves, but there is an amount of milk that is "lost". Fröberg et al. (2005) and Barth (2020) also reported that the reduction in milk volume collected at the milking parlour cannot be explained solely by the milk ingested by calves. The oxytocin release at milking is less marked when cows suckle their calves, either when suckling occurs just before milking (Lupoli et., 2001) or after milking (de Passillé et al., 2008). This could explain partly why all suckling cows in our experiments released less milk at the milking parlour even when the calves drank very little milk or when only female calves are kept with their dams. Nevertheless, the decreased total milk production occurred mainly at the time of the lactation peak, which was absent in Before-cows and Mixed-cows and reduced in Dam-cows, whereas interestingly, total milk production of Control- and Dam-cows, in Trial 2 and 3, was similar after Week 8 although calves were still suckling.

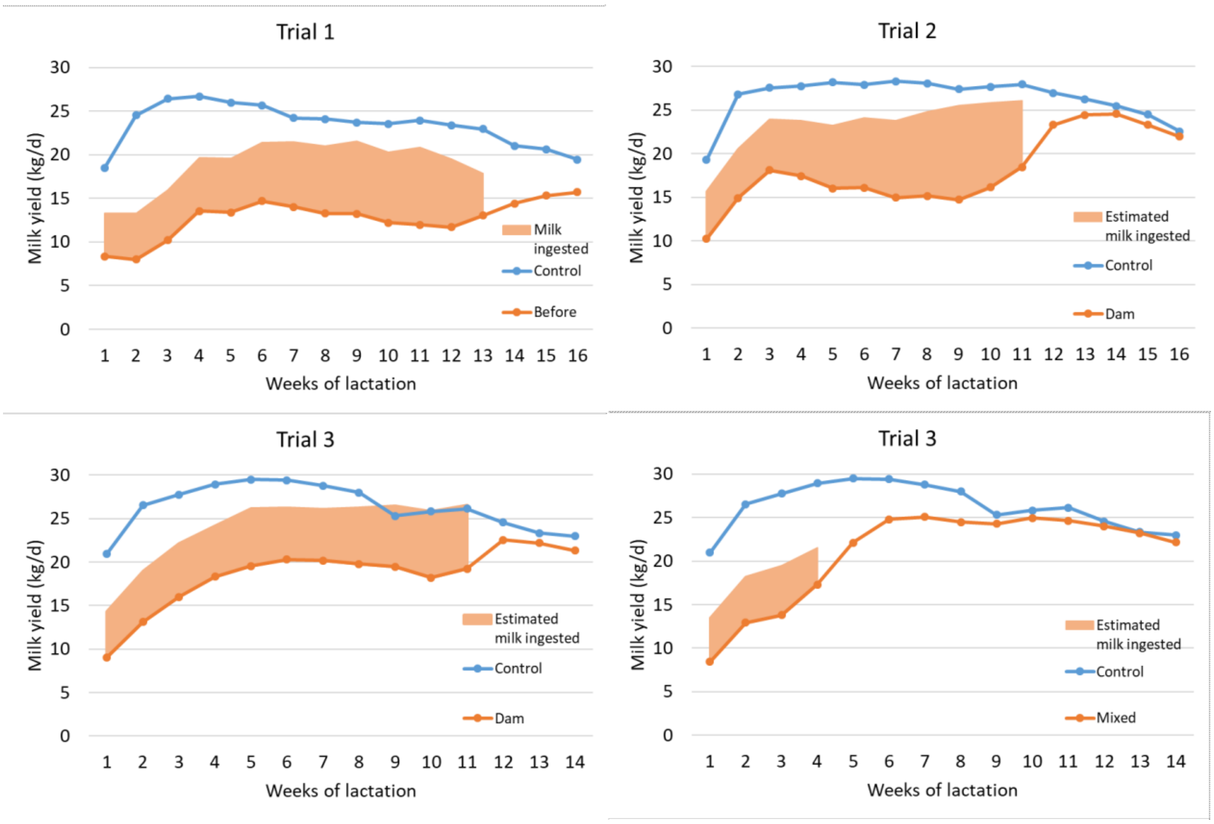


Figure 6.7. Total milk production (milked milk + milk ingested by calves) of suckled cows (Before-, Mixed and Dam-cows) in comparison to control-cows during suckling period in Trial 1, 2 and 3.

In all scenarios, milk composition was affected. Milk protein content was systematically higher in CCC cows (+1.0 to +3.0 g/kg; Table 6.3) compared to Control-cows and milk fat content was lower in CCC cows (-0.8 to -5.8 g/kg) except when calves drank the poor fat milk of the beginning of milking (+3.2 g/kg, Before group). As reported in literature, it is known that milk fat content decreases when calves suckle their dam after milking (Bar-Peled et al., 1995; Fröberg et al., 2007; Fröberg et al., 2005; Margerison et al., 2002; Mendoza et al., 2010) and may increase when they suckle before milking (Tesorero et al., 2001). Indeed, when suckling occurs after milking, calves mainly consume the residual milk, which has a high fat content, whereas when suckling occurs before milking, calves suckle the cisternal milk, which has a low fat content (Fröberg et al., 2007; Tesorero et al., 2001).

Contrary to fat, milk protein content appears to be less affected by suckling (Bar-Peled et al., 1995; Fröberg et al., 2007; Fröberg et al., 2005; Kišac et al., 2011; Mendoza et al., 2010) as milk protein content does not change during milking. However, few studies found an increase of milk protein content in the milked milk (Barth, 2020; Boden & Leaver, 1994; Margerison et al., 2002). Normally, milk protein content increases when the energy balance of the cows is higher (Coulon & Rémond, 1991). For this reason, we hypothesised that, since suckling cows produced less total (suckled + milked) milk and since they probably had the same feed intake as Control-cows, their energy balance was higher than Controls. This hypothesis is supported by the higher BCS at weaning of suckled cows in comparison to controls cows in Trials 2 and 3. However, the analyses of the blood plasma metabolites made in this study (Chapter 3) did not confirm our hypothesis. The higher milk protein content could therefore be also the consequence of a dilution effect as the total milk production of CCC cows was lower on average from Week 1 to Week 8 in the three trails.

Finally, there were no significant differences in milk SCC between CCC and Control animals in any of the three trials. Literature reports that suckling can improve the udder health and decrease milk SCC during the suckling period (Fröberg et al., 2005; Margerison et al., 2002; Krohn, 2001) or have no effect (Beaver et al., 2019; Johnsen et al., 2016). Our results confirmed that suckling did not affected milk SCC. Neverheless, altough not significant, milk SCC were numerically slightly higher in suckled cows than in Controls in the three trials even if milk SCC remain low. Moreover, as reported by De Oliveira et al. (2020), the somatic cell count increases during milking or suckling and therefore the time of sampling and the milk fraction sampled could bias the results. This suggests that further studies are needed to investigate on the consequences of suckling on udder health.

In conclusion, a short-time CCC immediately before milking, strongly decreases the amount of sellable milk but improves the milk composition at parlour. While, in contrast to our hypothesis, reducing the duration of day-time CCC to 4 weeks (vs. until weaning) did not strongly reduce the loss of sellable milk and, on the contrary, the reduction in total milk production was higher than a day-time CCC until weaning. Anyway, a four-weeks day-time contact less affects the milk composition at parlour than a day-time CCC until weaning. Finally, we can also state that the reduction in saleable milk and in total milk production, in a day-time CCC until weaning, is similar whether all calves are kept with their dams or that only female calves are kept after 4 weeks of age.

6.2.2. Consequences of cow-calf contact on cows' reproductive performances

We have collected reproduction data from the three trials with the aim to check if the reproduction performances of cows were or were not affected by calves suckling. Previous studies, reviewed by Krohn (2001), showed that suckling postpones normal follicle activity and heat behavior until after weaning, which results in a significant lengthening of the post-partum anoestrus interval. Nevertheless, Krohn (2001) concluded that suckling does not affect the number of empty days nor the calving interval because of a relatively higher fertility of the cows when the calves are removed.

After checking, eliminating and correcting some data, we performed Chi-square tests on binary data (yes/no) of "pregnancy" and "new calving" [total data, by type of suckling (short duration/day) and by parity (primiparous/multiparous]) as well as analysis of variance (SAS GLM) for the different intervals, considering type of suckling and parity in the model. We found no significant difference between the cows that suckle their calves and those that did not. The proportion of pregnant cows and the new calvings were similar in Control and Dam cows (Figure 6.8A) as well as the intervals between calving and 1st insemination, calving and conception and calving and next calving. The interval between calving and 1st insemination was longer in primiparous than in multiparous cows (Table 6.4 and Figure 6.8B) and interestingly we found a significant interaction between groups and parity for the intervals between calving and conception and calving interval (Table 6.4 and Figure 6.8C and 6.8D).

Table 6.4. Effect of CCC practice and parity on cow reproductive performances.

	Group			*P-Value*		
	Control	Dam	SEM	Group	Parity	Group × Parity
Interval calving - 1st insemination (Days)	91.9	88.3	0.59	0.59	0.01	0.47
Interval calving - conception (Days)	117	109	0.32	0.32	0.35	0.02
Calving interval (Days)	394	387	0.39	0.39	0.61	0.02

Therefore, the presence of calves seems to improve the reproductive performance of primiparous cows. In particular, the intervals from calving to conception (Figure 6.8C) and calving interval (Figure 6.8D) were reduced by 30 days in Dam primiparous cows in comparison to Control- primiparous cows while they were not modified for multiparous cows.

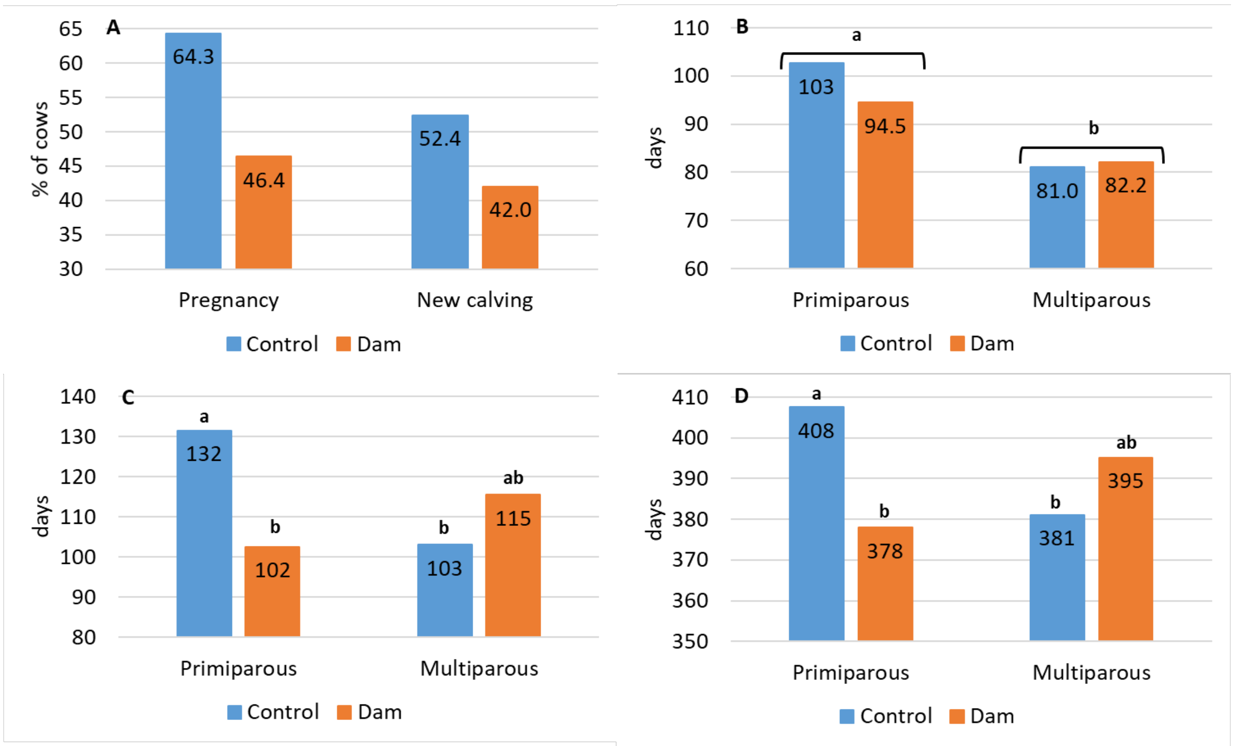


Figure 6.8. Performances of reproduction (proportion of pregnant cows and new calvings - A) in Control (n= 42) and Dam cows (Before, Dam2 and Dam3, n=69) and intervals from calving to first insemination (B), calving to conception (C) and calving interval (D) from of Control and Dam cows according to parity.

Considering these results, we could confirm the previous conclusions of Krohn (2001) that suckling does not impair calving interval. We could show as well that, on the contrary, suckling seems to improve the reproduction of primiparous cows, which is the most challenging in dairy farms. This result is therefore particularly interesting and it represents an original result that was never reported previously to our knowledge.

6.2.3. Consequences of cow-calf contact on calves' growth

Compared to Control-calves, calf growth rate until weaning was higher in Before- and Dam3-calves (+ 171 g/d and + 251 g/kg; Table 6.3) and was similar in Dam2- and Mixed-calves. As reported in Chapter 1, benefits on calves' growth depend on the type and the duration of CCC practice. Suckling before milking and partial-time CCC until weaning improve calves' growth, according to Roth et al. (2009) and Johnsen et al. (2015a). This benefit may come from the higher amounts of milk ingested or from the positive effect of suckling on calf metabolism (Uvnäs-Moberg et al., 2001). However, the comparison between trials is not easy to make because it also depends on the growth and on the amount of feed distributed to Control-calves, which in the case of Trial 1 was lower than in Trials 2 and 3. Nevertheless, we can still affirm that a day-time CCC for few weeks (Mixed-calves) had no beneficial effects on calves' growth.

In all CCC practices we found that weaning after three months induces a strong slowdown of calves' growth. The drop in growth was also observed, to a lesser extent in Control-calves for which milk removal had been more progressive, allowing them to get accustomed to a solid diet. It thus appears unlikely that nutritional aspects alone fully explain the observed post-weaning growth reduction. The stress experienced at weaning may also affect growth. Most studies on calves growth after weaning found that weaning has a stronger impact on daily weight gain of suckling calves than of artificially reared calves (Bar-Peled et al., 1997; de Passillé et al., 2008; Hepola et al., 2007). However, this disadvantage can be reduced if the weaned calves are older (12/13 weeks) as they are already accustomed to solid food in the diet and thus the negative effects on weight loss can be limited (de Passillé et al., 2011). Finally, we can state that a short-time CCC before milking and a day-time CCC until weaning had beneficial effects on calves' growth compared to artificially reared calves, consistent with Roth et al. (2009) and Fröberg et al. (2011).

6.2.4. Consequences of cow-calf contact on cows' and calves' health

During the milk feeding period calves are more exposed to respiratory diseases and diarrhea. As discussed in Chapter 1, a common belief is that separating a calf at birth limits the risk of transmission of diseases and is better for both calf and cow health. Artificial feeding of calves is thought to better control the quantity and the quality of colostrum ingested allowing a better transfer of maternal immunoglobulins to the calf (Beaver et al., 2019). To study the effect of CCC practices on the passive transfer of immunity from cows to calves and antibodies in suckling cows' milk, we analyzed the IgG concentrations in colostrum, milk and serum of calves in Trial 3 (see Chapter 3). We concluded that the passive immunity transfer from cows to calves was adequate in all groups and that CCC practices (Dam and Mixed groups) had no adverse effect on neonatal calves' immunity and no effect on the further build-up of the active immune defense during the pre-weaning period.

Studies that investigated the transmission of pathogens from adult animals to calves are limited but we did not consider this aspect in our study, because the number of animals in the trials would have been to limited to draw robust conclusions. The contact of calves with adult animals in a shared environment (indoor housing or early access to pasture) during the milk feeding period can lead to pathogen transmission, as the husbandry of different aged cattle is considered a risk of transmission (Johnsen et al., 2016). The few studies that are available in the literature investigated disease transmission in farming systems with foster cows. Köllmann et al. (2021) studied the transmission of *P. multocida* and *S. aureus* trough suckling and concluded that it is very likely. They suggested that to reduce this risk, in addition to sufficient intake of colostrum it is important that cow accept foster calves to ensure sufficient milk intake. Constancis et al. (2022) studied parasites contamination at first grazing season of calves reared with foster cows and they concluded that it is possible to turn out calves at an early age in a protective grazing management system during the first grazing season. They reported that potential risk factors of gastrointestinal infection for calves include the ratio of calves per foster cow and the grazing season duration, as the presence of adult animals dilutes the risk of gastrointestinal infection. On beef farms, suckling calves are generally in contact with the herd during early gestation, prior to the time when the bovine fetus develops a competent immune system (Larson et al., 2004). Consequently, persistently infected (PI) suckling calves are considered the primary source of Bovine Viral Diarrhea (BVD) infection on breeding farms causing decreased pregnancy rates, pregnancy loss, pre-weaning mortality and induction of PI calves in the next generation (McClurkin et al., 1984; Wittum et al., 2001). It has been shown that adequate intake of colostrum from BVD-seropositive dams and vaccination against BVD of young calves can reduce clinical disease and mortality compared with colostrum-deprived and unvaccinated calves (Cortese et al., 1998). The impact of environmental and management factors also play a major role in BVD contamination (Lundborg et al., 2005). The authors of this study recommend rearing calves separated from outside walls and kept draught-free to reduce the incidence of diarrhoea or other infectious diseases.

Considering that we did not find any difference between groups in any of the 3 trials because of the small number of animals in each group, we performed Chi-square tests on binary data (yes/no) of animal health disorders registered in the three trials. This allowed getting an overview of the effect of different CCC on cows' and calves' health. All the health disorders correspond to disorders that required a medical treatment. They were sorted into reproductive disorders (metritis, retention of the placental membrane, ovarian cysts, and vaginitis) and non-reproductive disorders (mastitis, milk fever, lameness, etc.) for cows, and respiratory disorders (runny nose, coughing, dyspnoea, etc.) and non-respiratory disorders (diarrhoea, umbilical infection, etc.) for calves (Table 6.5).

Table 6.5. Chi-squared test on the number of cows and calves attending health disorders in Control (n= 42) and Dam groups (Before, Dam2 and Dam3, n=42). Health disorders are classified into *Reproductive disorders* and *Non-reproductive disorders* for cows, and *Respiratory disorders* and *Non-respiratory disorders* for calves.

			Real		Expected		Chi-squared test
			Control	Dam	Control	Dam	
Cows	Reproductive	yes	7	9	8	8	0.58
		no	35	33	34	34	
	Non-reproductive	yes	16	14	15	15	0.65
		no	26	28	27	27	
Calves	Respiratory	yes	6	13	10	10	0.06
		no	31	24	28	28	
	Non-respiratory	yes	7	3	5	5	0.18
		no	30	34	32	32	

As reported in Table 6.5, the frequency of health disorders was not significantly different between groups of cows and calves in the three trials. Our results are in contrast with some authors in literature, who reported that dam presence had beneficial effects on calves' health (Weary & Chua, 2000; Metz, 1987; Boonbrahm et al., 2004). However, according to Beaver et al. (2019) who reviewed the available literature and found no evidence that suckling has negative effects on health, we can conclude that regardless of CCC practice, suckling did not affect the occurrence of animals' health disorders.

In conclusion, allowing calves to suckle their dam for 20 min immediately before milking allows calves to cover their nutritional requirements but affects milk yield to an extent that seems not compatible with dairy production. Allowing cows to suckle their calves for a long period (6-9h/day) between morning and evening milking for 4 weeks seems to affect calves' growth and to reduce saleable milk even after the separation of the calves. Finally, allowing cows to suckle their calves for a long period (6-9h/day) between morning and evening milking until weaning seems to offer a good compromise between satisfying the calves' nutritional requirements and limiting the reduction in saleable milk from the cows.

Therefore, considering all our results, the best compromise between cow milk yield and calf growth and welfare is a long period of CCC (6 to 9 h) between the morning and evening milkings until weaning. Suckling did not affect cows reproductive performances or animals' health. However, abrupt weaning stresses both cows and calves even if CCC has been restricted before separation.

6.3. Economic viability of CCC practices

Early cow and calf separation is known to be a contentious issue world-wide (Beaver et al., 2020). Considering the results presented so far, is it then possible to implement economically viable CCC systems?

Implementing CCC systems requires farmers to rethink daily practices and long-term priorities compared to systems where calves are separated from their dams at birth and their interaction with

animals must be different when calves are reared with cows and not in calves' pens (Ferneborg et al., 2020).
Apart from giving the animals increased opportunity to express their natural behavior, when keeping a calf with its dam, the dam provides the care necessary for its survival and feeding so the farmer can review the workload he previously employed to calves' care into other tasks (Fröberg et al., 2011). According with Mendoza et al. (2010), restricted suckling may be a simple and viable alternative for dairy farmers interested in obtaining heavier male or female calves at weaning without significantly affecting the management of the herd (Kišac et al., 2011). Moreover, increased weight gain of heifers can be an advantage in reducing age at first calving (Shamay et al., 2005).
Another aspect to consider is the human-animal relationship (HAR) as in CCC practices it may be more difficult for caretakers to establish a good HAR due to a lack of contact opportunities (Johnsen et al., 2016). CCC calves are not necessarily fed by human caretakers and may stay mainly in the cow barn with the risk to receive less positive human contact and relatively more negative treatment, such as ear tagging and disbudding (De Oliveira et al., 2020). Waiblinger et al. (2020) found that human contact associated with feeding (bottle feeding or lactation assistance) in both artificial and CCC practices influenced the release of several hormones involved in filial bonding and thus can support a positive relationship with humans. They concluded that close contact with humans during lactation in the first days or weeks of life in cow-calf contact systems can improve calves' relationship with humans.
Furthermore, Johnsen et al. (2016) suggests that, among suckling practices, a daytime CCC allows calves to get used to daily separation from the dam and to being handled by humans.
Studies on the long-term consequences of CCC practices on HAR are not yet available, and more experimental and observational studies are needed to enhance the knowledge on these issues (De Oliveira et al., 2020).
Moreover, in our study we did not take under consideration the cowshed design (see Chapter 5), and it is important to consider that modern farm buildings today are not always designed for rearing cows and calves together (Asheim et al., 2016). Slatted floors conceived for cows could be a problem for calves. Therefore, some farmers may need to invest in additional buildings and interiors if they consider having cows and calves together in a loose-housing system.
Furthermore, our study focused on three dairy farming systems that are representative of specialised dairy systems existing in France and was based on data collected in this thesis. We decided to study suckling practices that were closer to current realities, i.e. all male calves were sold at 3 weeks of age and cow-calf contact was allowed only during the first weeks of life, as it is usual in dairy farms that practice natural suckling in France (Michaud et al., 2018). We therefore only considered data on the dams of the female calves that will become the future heifers, and data on these female calves.
It is important to state that it is not easy to predict economic changes on a theoretical basis in systems involving CCC, because there is a large variety of rearing systems with as many number of interaction factors, even if there are common factors among the different systems such as the reduction in the amount of saleable milk or the increased growth rate of calves. We chose to apply a modeling method on case-studies, but if CCC practices will widely develop we can consider studying their economic consequences from surveys. Further investigations should be implemented on the economic impact of CCC systems by performing sensitivity analysis on milk price and on workforce, and to assess how work organization would be affected by such practices. Moreover, further studies of economic consequences of CCC systems on other EU countries are needed to sketch a wider overview of this economic analysis.
Neverthless, the analysis we conducted comparing the economic impact of two types of CCC practices on the farm's gross operating profit (Chapter 5) represents one of the few studies performed on this topic to date. We concluded from these simulations that the short-time daily contact system is associated to a reduction of net farm income per worker varying from – 6 k€/year in the extensive

system to - 11 k€/ year for the intensive and organic systems. On the opposite, a day-time contact system for 12 weeks can be economically viable (+0.5 k€ per worker) both in an intensive and in an organic farming system, since, despite the loss of milk due to suckling, there is also a reduction and an improvement of working conditions, associated with a better growth of calves. Positive aspects associated with a CCC practice could be a higher price paid for male calves due to the higher weight gain reach after the suckling period, but in our study we did not took into account male calves sold at 3 weeks of age. Indeed, the application of future labelling initiatives on the price paid for calves reared with their dams could encourage this practice, even if it seems that higher prices are already applied for the sale of calves reared in this way (Bickelhaupt & Verwer, 2013). Finally, the reduction in the milk price is marginal, so this practice would be economically viable if we assume the application of a label on milk of this kind of system.

As reported by Janssen et al. (2016) in a meta-analysis of consumer studies, generally consumers have positive attitudes towards animal welfare-friendly husbandry systems and they are willing to pay more for meat and milk produced in such systems. CCC systems, as other aspects related to animal welfare, could be attractive for a growing number of consumers from specific market segments (Carlucci et al., 2009), including those who are considering avoiding consumption of dairy products because of animal welfare concerns (Ferneborg et al., 2020).

In the last few years, the number of dairy farms that integrate the rearing of dairy calves with their dams or with foster cows has been growing, with the aim of creating systems more respectful of the animals and responding to the demand of society. Some examples of such kinds of dairy farms in different European countries are provided in Appendix 1.

In conclusion, we can state that the implementation in dairy systems of a day-time CCC practice during 12 weeks could be an economically viable option as the loss of saleable milk due to suckling is compensated by improved calf growth and reduced workload. Moreover, the application of labels that identify “animal welfare” practices or “husbandry systems” traceability should be investigated to have the opportunity to generate an added value of this kind of practice.

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General conclusion

To meet the growing public interest on livestock animals' welfare and farmers' questioning the sustainability of cow-calf contact (CCC) practices, this thesis showed that it is possible to implement CCC practices in a modern dairy farming system. Our aim was to find the CCC practice that provides the best compromise between animal welfare and animal production performances.
A short-time CCC immediately before weaning has a positive effect on calves growth and milk composition, nevertheless it induces a significant reduction in saleable milk. A day-time CCC for few weeks slightly reduce the milk losses and cows' distress. However, it induces two periods of stress for calves (separation then weaning) instead of one (simultaneous separation and weaning), without benefit on calves' growth and milk composition.
A day-time contact until weaning (11 weeks) also affects the milk yield and composition at parlour. The loss of saleable milk is due to more milk drunk by calves but also to a removal of the lactation peak. Changes in milk composition, in particular the reduction in milk fat content, can affect the milk price. However, the economic analysis shows that these losses can be compensated by the reduction of workload and the improved calves' growth.
In conclusion, we can say that the best compromise in terms of animal performance, animals' behavior and health and economic viability is a day-time CCC rearing system until weaning.
Allowing cows and calves to be in contact half a day best mimics the natural situation and allows for maternal behaviors. Cow-calf contact provide the behavioral and physiological development of both. Cows can express their maternal behavior and their natural need to nurse and care of the calf, while calves may have the opportunity to learn from adult individuals about social rules, social behaviors and may become more confident towards their surroundings. Keeping calves with their dams allows performing natural behavior such as suckling and social bonding with dam, learning to eat roughage earlier and having social contact with other calves and cows.
Suckling during the colostrum period did not affect the passive transfer of immunity from cows to calves, cows' reproductive performances or animals' health.
Restricted daily contact allows animals to get used to separation, but abrupt separation or weaning is anyway a welfare problem that must be solved by implementing gradual weaning practices.
Even if weaning distress is stressful for both cows and calves, the dam presence until weaning reduces the chronic stress in calves during the pre-weaning period.

Ultimately, this thesis contributed to improving the knowledge about CCC dairy systems, by finding innovative results that may contribute to the spread of these practices in more production realities. The references provided by this thesis are important, but the experimental results obtained here need to be validated by on-farm studies questioning the technical and economic feasibility of implementing these practices in a wide variety of production systems, from the most intensive to the most extensive. At present, all the conditions for the development of such systems seem to be in place. There is an awareness and demand from consumers, and from farmers' perspective, our results have demonstrated the feasibility of the implementation of such practices. The progress made in the recent years attest that both consumers and farmers are interested in the development of these practices. They will contribute to the desired improvement of livestock rearing conditions so that natural needs and welfare of animals are fully respected.

Appendix 1

Some examples of dairy farms that practice CCC practices in different European countries:

- "La Bille Bleu" in Pays de la Loire' region (France) where calves are reared minimum 7.5 months under their dam or a foster cow, they are slaughtered on the farm. Marketing: cheese and meat veal. In their website they reported: *"The introduction of a dam-foster cow system in dairy farming offers many solutions in terms of animal welfare, rearing management, economic opportunities and coherence of the sector. It is an opportunity to meet the needs of all the stakeholders in the dairy industry, from the animals to the consumer, via the farmer."* (https://www.labillebleue.farm/France) (Figure);
- "Società Agricola biodinamica San Michele" in Veneto region (Italy), where calves are reared for 90-100 days under their dam or a foster cow. Marketing: Milk for processing into organic cheese in the farm and milk for beverage (https://www.biodinamicasanmichele.it/);
- "Hofgut Rengoldshausen" Überlingen, in Germany where calves are reared under their dam or a foster cow until 18 weeks of age and then gradually weaned. Direct marketing of meat (50-60 %) and Demeter certified raw milk (https://www.rengo.de/);
- "Randenhof" Siblingen, in Switzerland, where calves are reared under their dam or a foster cow until 16 weeks of age. Marketing: Direct marketing and distribution channel through Demeter to specialized organic outlets (https://www.randenhof.ch/).

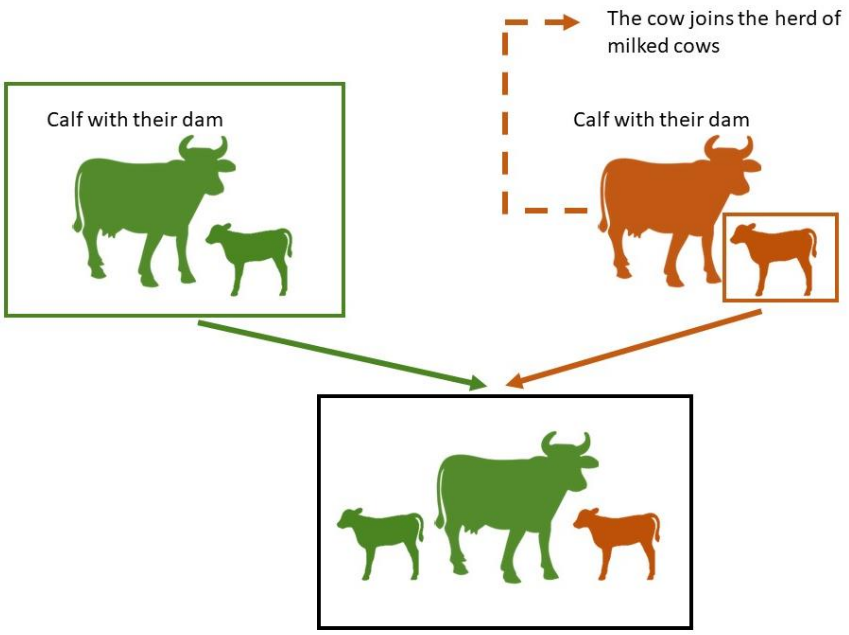

Figure. Illustration of calves' rearing system under their dam or a foster cow in « La Bille Bleu » farm's website.

List of Publications

Publications in International Journals

Alessandra Nicolao, Mauro Coppa, Matthieu Bouchon, Enrico Sturaro, Dominique Pomiès, Bruno Martin, Madeline Koczura. *Early life dam-calf contact and grazing experience shortly influences post-weaning behavior and herbage selection of dairy calves.* Published on *Frontiers in Veterinary Science,* on December 7, 2020.

Alessandra Nicolao, Bruno Martin, Matthieu Bouchon, Enrico Sturaro, Isabelle Veissier and Dominique Pomiès. *A suckling dairy system is viable if calves are allowed to suckle their mothers for a long time between the two daily milkings, but stress is important for cows at weaning separation.* Submitted to *Animal,* on December 27, 2021.

Publications in Proceedings of International Conferences

Alessandra Nicolao, Bruno Martin, Matthieu Bouchon, Enrico Sturaro, Dominique Pomiès. *Suckling of dairy calves by their dams: consequences on animal performances, behavior and welfare.* – EAAP, August 2019 Ghent, Belgium.

Alessandra Nicolao, Madeline Koczura, Anna Mathieu, Matthieu Bouchon, Enrico Sturaro, Bruno Martin, Dominique Pomiès. *Which compromise between milk production and cow-calf contact in dairy systems?* - IAHA Virtual Video Pre-Conference on "Organic Animal Husbandry systems challenges, performance and potentials", September 21-22, 2020.

Alessandra Nicolao, Madeline Koczura, Matthieu Bouchon, Enrico Sturaro, Dominique Pomiès, Bruno Martin, Mauro Coppa. *Is dairy calves grazing behavior influenced by cow-calf contact experience?* - IAHA Virtual Video Pre-Conference on "Organic Animal Husbandry systems challenges, performance and potentials", September 21-22, 2020.

Alessandra Nicolao, Claire Mosnier, Bruno Martin , Dominique Pomies , Enrico Sturaro. *Suckling of dairy calves by their dams: trade-off between ethical and economic sustainability.* - ASPA 2021 "24th Congress of the Animal Science and Production Association", September 21-24, 2021 Padova.

Oral presentations

Alessandra Nicolao, Madeline Koczura, Anna Mathieu, Matthieu Bouchon, Enrico Sturaro, Bruno Martin, Dominique Pomiès. *Which compromise between milk production and cow-calf contact in dairy systems?* - IAHA Virtual Video Pre-Conference on "Organic Animal Husbandry systems challenges, performance and potentials", September 21-22, 2020.

Alessandra Nicolao, Claire Mosnier, Bruno Martin , Dominique Pomies , Enrico Sturaro. *Suckling of dairy calves by their dams: trade-off between ethical and economic sustainability*. - ASPA 2021 "24th Congress of the Animal Science and Production Association", September 21-24, 2021 Padova.

Acknowledgments

Grazie ad Enrico per aver creduto in me e per avermi scelta come tua dottoranda, grazie per la pazienza che hai avuto in questi anni, è stata l'esperienza più importante della mia vita e ti ringrazio per avermi dato questa opportunità speciale.

Je remercie Bruno, Dominique et Isabelle. Bruno, merci de m'avoir accompagnée pendant ces trois années, ce fut un parcours tumultueux mais formateur, si bien que je suis heureuse de l'avoir poursuivi jusqu'au bout. Tu as été un soutien important, je te remercie d'avoir été à mes côtés, pour la patience et la gentillesse dont tu as fait preuve pendant ces années. Je te remercie aussi pour l'aide concrète que tu m'as apportée dans la rédaction de cette thèse.
Dominique merci, car sans toi je ne serais pas là aujourd'hui. Je te remercie pour tout le temps que tu m'as consacré, pour avoir ouvert les portes de chez toi et m'avoir permis de travailler avec toi. Tu as réussi à me redonner le courage et la confiance que j'avais perdus dans les moments difficiles et cela m'a permis de ne pas abandonner et d'aller jusqu'au bout. Je t'en serai éternellement reconnaissante.
Isabelle, merci car même si tu ne faisais pas officiellement partie de mon encadrement, tu as été présente pendant mon parcours, toujours disponible et à l'écoute. J'ai trouvé en toi du soutien, de la disponibilité et de la gentillesse, tu as beaucoup contribué à la réussite de cette thèse.

Je tiens à remercier Marcenat et toutes les personnes avec lesquelles j'ai travaillé pendant les essais, vous étiez un peu comme ma deuxième famille et je vous remercie pour tous les bons moments que l'on a partagés ensemble. Je veux aussi remercier Anna, Elisa et Madeline parce que vous avez fait de ces mois passés à Marcenat une expérience unique, merci pour les rires, les apéritifs chez Simone, les soirées cinéma et burgers, la complicité et les heures passées sous le soleil à observer les veaux et les batailles d'eau dans la salle de traite.

Grazie ai miei amici di Padova, Celio, Giulia, Enrico e Nicolò. Grazie a voi i mesi passati a Padova sono stati pieni di spensieratezza, risate e tanti tanti spritz. Mi siete mancati negli ultimi tempi, grazie per i bei momenti passati insieme.

Merci à la Pauline, la Hélène et Ruben. Vous avez fait partie de ma vie lors de ma première expérience en France et vous ne l'avez jamais quittée. Vous avez été un point de repère important durant ces années, merci pour tout ce que nous avons partagé ensemble, j'espère que de nombreuses autres belles expériences nous attendent à l'avenir.

Grazie a Louise e a Ruggero. Louise, j'ai découvert en toi une amie sincère au cœur immense. Merci pour toutes les heures qu'on a passées à bavarder et à philosopher sur la vie. Ruggi, siamo diventati amici alla velocità della luce, è stato bellissimo condividere con te i mesi che hai trascorso qui a Clermont ma anche quelli in cui il covid e la vita ci hanno portato a vivere lontani. Spero che la mia strada mi porti ancora ad incrociare le vostre vite.

Merci à la coloc de Delille, Vanessa et Pauline, et Amandine et Mélanie. Vanessa et Pauline vous avez accompagné mon quotidien depuis mon arrivée en France, vous m'avez fait découvrir ce que signifie vivre en coloc et cela a été une merveilleuse expérience avec vous. Amandine et Mélanie merci d'avoir partagé le confinement avec moi. Ça a été, malgré le contexte difficile, un des plus beaux moments de ces années.

Grazie ai miei amici storici, Babu, Fra, Angela e Richi, e Silvia. In tutti questi anni di lontananza non vi ho mai sentiti distanti, ogni ritorno a casa è come se non fossi mai partita. Sono molto fortunata ad avervi e vi voglio un bene immenso. Grazie per esserci sempre.

Merci alla Manone, je sais que je pourrai toujours compter sur toi. Merci de m'avoir soutenue dans les moments difficiles mais surtout merci pour tous les moments heureux et les belles expériences que l’on a partagés ensemble. Merci à Remino, Chiara, Ben et Djoby parce que vous êtes des amis spéciaux, je serai toujours reconnaissant de vous avoir dans ma vie. Merci à Lucas et Mélo, même si vous étiez physiquement loin je n’ai jamais vraiment ressenti la distance avec vous. Merci pour l'amour que vous tous m'avez apporté pendant ces années.

Merci à Jimmy. Mon compagnon de vie. Pendant toutes ces années, tu as partagé avec moi tous les bons et les mauvais moments. Ta présence a été fondamentale dans ma vie. Merci du fond de mon cœur, si je suis arrivée jusqu'ici aujourd'hui, c'est principalement grâce à toi.
Merci aussi à ta famille, à Mélody pour les beaux moments que l’on a partagés pendant les mois de cohabitation, ça m’a permis de mieux te connaître et de t'aimer comme une sœur. Merci à Louise et Yohan, pour l'infinie gentillesse et l'amour que vous me transmettez constamment.

Grazie alla mia piccola Olimpia, il tuo arrivo nella mia vita ha cambiato ogni cosa e te ne sarò eternalemente grata.

Infine grazie alla mia famiglia. Mamma, papà e Franci, senza di voi non sarei quella che sono oggi. Grazie per supportarmi in ogni mia scelta, per essere sempre presenti e per amarmi senza limiti. Questo traguardo lo dedico a voi.

Et merci à mes petits veaux de Marcenat.