Applied Animal Behaviour Science 252 (2022) 105644

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

journal homepage: www.elsevier.com/locate/applanim

Effects of twice a day teat bucket feeding compared to twice a day mother suckling on behaviour, health traits and blood immune parameters in dairy calves and immune parameters in cow's milk

Anna Bieber [a,*], Michael Walkenhorst [a], Rennie Eppenstein [a], Johanna K. Probst [a,1], Susann Thüer [a], Cem Baki [a], Bruno Martin [b], Anet Spengler Neff [a]

[a] Department of Livestock Sciences, Research Institute of Organic Agriculture (FiBL), Ackerstrasse 113, CH-5070 Frick, Switzerland

[b] Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, 63122 Saint-Genès-Champanelle, France

ARTICLE INFO

Keywords:
Cow-calf contact
Dam rearing
Calf health
Animal behaviour
Animal welfare

ABSTRACT

Early separation of cow and calf is still common practice in dairy production systems, but interest in calf rearing with cow contact has been constantly increasing in recent years. We tested the hypothesis that calves suckling their mothers twice a day would benefit with regard to behaviour and health traits until four months of age, when compared to twice daily teat bucket fed calves, fed with comparable milk amounts. Moreover, the effect of calf contact beyond the colostrum period on immunoglobulin G (IgG) and lactoferrin content in cow's milk was investigated in weeks 3, 8, 12 and 16 post partum. We conducted on-farm trials on two organic farms from 2018 to 2020, where we randomly assigned the new born calves by sex and parity status of the mother (primi- or multiparous) to either teat bucket feeding (BF, n = 30 cow-calf couples, 2 ×3–5 L/d warmed bulk milk) or mother suckling (MS, n = 28 cow-calf couples, 2 ×30 min contact after milking). MS calves performed less cross-sucking, but manipulations of objects did not differ between feeding treatments nor did avoidance distance towards humans. Clinical scores on vitality, body condition, indicators for diarrhoea and respiratory disorders, and number of medical treatments differed between farms, but not between feeding treatments. Lactate level (stress indicator) revealed contradictory results between farms (farm 1: BF>MS, farm 2: BF≤MS). Glucose content (indicator of energy level) and packed cell volume (low values indicating anaemia) were higher in MS compared to BF calves. No difference between feeding treatments was found with regard to the immune status indicators mean total protein and IgG content in calf serum. Consistently, average IgG content in cow's milk did not differ between cows with or without calf contact and showed a clear decrease with time. The variability of lactoferrin content in milk was higher in cows with calf contact, but its average did not differ between feeding treatments nor did it change with time. We conclude that even part-time mother contact twice daily is a means to reduce abnormal behaviour, i.e. cross-sucking, as it satisfies sucking needs of calves better than teat bucket feeding. Although health traits did mostly not differ between feeding treatments, differences between farms underline the influence of management factors on calf health. Cow-calf contact twice daily had no effect on the build-up of the active immune defence until the age of four months.

1. Introduction

Separation of cow and calf within less than one day is still common practice in dairy production systems (e.g. Kälber and Barth, 2014; Meagher et al., 2019). Reasons for early separation are increasing the amount of saleable milk, controlling colostrum intake, preventing milk ejection problems, reducing separation stress (reviewed by Flower and Weary, 2003), and prevention of pathogen transfer from cow to calf (e.g. Beaver et al., 2019; Busch et al., 2017).

Early separation of mother and calf is seen rather critically by consumers (reviewed by Placzek et al., 2021). For farmers animal health, labour satisfaction and animal welfare aspects are major drivers to practice cow-calf contact systems (Eriksson et al., 2021; Vaarst et al., 2020).

* Corresponding author.
E-mail address: anna.bieber@fibl.org (A. Bieber).

1 PROROSS, Im Holz 44, 79777 Ühlingen-Birkendorf, Germany.

https://doi.org/10.1016/j.applanim.2022.105644
Received 22 December 2021; Received in revised form 27 April 2022; Accepted 2 May 2022
Available online 5 May 2022

Number of cow-calf contact (CCC) farms has increased. They differ with regard to (a) total duration of contact allowance (days to months), (b) daily contact duration and time slots (whole-day, part-time, i.e. either restricted to several short times a day or during daytime/ night time), and (c) the animal being suckled (dam, foster cow, both in succession) (Eriksson et al., 2021; Kälber and Barth, 2014; Johnsen et al., 2016).

In terms of behaviour, CCC systems enable to perform natural behaviour and reduce abnormal oral behaviours in calves (e.g. Fröberg et al., 2008; Kälber and Barth, 2014; Meagher et al., 2019), while weaning and separation stress are reported to be challenging in CCC systems (e.g. Eriksson et al., 2021; Johnsen et al., 2016).

With the exception of two studies, a systematic literature review on CCC, found no (n = 8) or a positive effect (n = 6) of CCC on diarrhoea in calves (Beaver et al., 2019). The review found no effect of CCC on respiratory risks and came to the overall conclusion that evidence for early separation as a means of preventing disease transmission is lacking (Beaver et al., 2019). Additionally, a most recent review on calf health confirms that "there is no evidence that early cow-calf separation is beneficial for the health of calf or cow" (Lorenz, 2021).

Benefits of CCC systems may be mainly due to the increased milk consumption linked to these systems (e.g. Johnsen et al., 2016; Mendoza et al., 2010), as also demonstrated by higher weight gains compared to artificially fed calves (e.g. Bar-Peled et al., 1997; Mendoza et al., 2010; Roth et al., 2009). One study attributed higher weight gains to the presence of the mother, even when calves were prevented from suckling (Krohn et al., 1999). In order to investigate the effects of CCC beyond effects of higher milk intake, a comparable milk supply was aimed for in both feeding treatments in the present study.

Results on passive immune transfer through suckling are contradictory: some studies report that dam-suckling calves have higher levels of immunoglobulin G (IgG) absorption and serum IgG concentrations than calves bottle-fed colostrum (e.g. Selman et al., 1971; Stott et al., 1979), while others did not identify differences in IgG concentration in CCC systems (Hillmann et al., 2019), or found higher failure rates for passive immune transfer in CCC systems (reviewed by Beaver et al., 2019). Consistently, the review of Beaver et al. (2019) emphasised that although the separation of cow and calf to ensure passive immune transfer is not evidence-based, CCC systems are not a substitute for a careful colostrum management.

CCC systems are associated with the risk to reduce animal-human relationship to mainly negative interactions (e.g. ear tagging, de Oliveira et al., 2020). Some farmers reported to have wilder calves after three to four weeks of CCC, while experiences regarding long-term effects of CCC on animal-human relationship are contradictory (Vaarst et al., 2020). We used the avoidance distance test (Waiblinger et al., 2003) to assess the impact of the two feeding treatments on the animal-human relationship.

Lactoferrin was studied due to its anti-microbial effects and its ability to modulate the adaptive immune system (reviewed by Superti, 2020). Calves receiving lactoferrin supplemented milk showed less days of disease with less serious cases of diarrhoea and higher weight gains (Prenner et al., 2007). In humans, lactoferrin is associated with prevention of gastrointestinal and respiratory pathogens in young children (Manzoni et al., 2018).

We aimed at testing the hypothesis that dairy calves reared with part-time mother contact twice daily would (a) show less cross-sucking and oral manipulations of objects, and (b) would benefit with regard to health traits compared to twice a day teat bucket fed calves receiving comparable amounts of milk. We wanted to find out whether feeding treatment affects avoidance distance in calves. Moreover, we investigated whether CCC beyond the colostrum period would have an effect on the production of IgG and lactoferrin in cow's milk.

2. Animals, material and methods

2.1. Farms and animals, ethics statement

We conducted on-farm trials on two commercial organic farms to compare the effects of feeding dairy calves by mother suckling (MS) or teat bucket feeding (BF) twice a day, respectively. We followed EU standards for the protection of animals used for scientific purposes (EU directive 2010/63/EU for animal experiments). The trials were approved by the Regierungspräsidum Freiburg, Germany (permission number G-1878) on farm 1, and by the cantonal veterinary office Aargau, Switzerland (permission number TV30875) on farm 2, respectively. All animal related procedures were conducted according to the European and Swiss legislations on animal welfare.

Farm 1 is a bio-dynamic farm with a dairy herd of 50 cows and one bull (farm size: 150 ha agricultural land, of which 85 ha are grassland and pasture, average altitude 640 m above sea level, average precipitation 1050 mm/a). The average milk yield is around 6000 kg/a. All calves are either fattened on the farm or kept as replacement heifers. Animals have been crossed back from Holstein Friesian to the local breed German Friesian Cattle (DSN) for more than 15 years using natural mating. DSN is an endangered dual-purpose breed (GEH, 2020), which is smaller and meatier than current modern Holstein Friesian cows.

On farm 1 cows were milked twice daily from 6:45–8:00 a.m. and from 5:45–7:00 p.m. in a tandem parlour with 7 places using Happel milking clusters (System Happel GmbH, Friesenried, Germany) at vacuum height of 41.0 kPa with automatic stripping arm and automatic cluster removal. Milking routine was performed by two different persons and comprised pre-milking, udder cleaning with disinfectant moist tissues, attaching and positioning of cluster. Udder and teats were controlled after cluster removal and only teats of cows without calf contact were dipped with Gelstadip. Milk samples of 50 ml were taken from the glass bell jar containing the total milking of an individual cow.

Farm 2 is certified organic according to the Swiss label organisation BioSuisse (farm size: 40 ha agricultural land, of which 31 ha are grassland and pasture, 8 ha are extensive pasture, average altitude 411 m above see level, with 1100 mm/a average precipitation). The dairy herd consists of 50–60 Swiss Fleckvieh dairy cows and one to three bulls. Swiss Fleckvieh is a dual-purpose breed with a strong emphasis on dairy traits. Nowadays the average percentage of Red Holstein blood in the population is 75% (https://www.swissherdbook.ch/unsere-rassen/swiss-fleckvieh/, accessed 07.01.2021). Average milk yield on farm 2 is approximately 5500 kg/a. Apart from replacement animals, calves are usually sold to another organic farm for fattening at the age of 5 months. Cows are mated mainly naturally, artificial insemination is also used.

On farm 2 two persons were milking twice daily from 5:30–6.45 a.m. and from 5.00–6.15 p.m., respectively. Cows were milked in a 2 × 5 tandem parlour (GEA Farm Technologies GmbH, Bönen, Germany) at vacuum height of 42.0 kPA with automatic cluster removal, which was individually switched on or off for each cow. Milking routine consisted of pre-milking, udder cleaning with wood wool, attaching and positioning of cluster. After cluster removal the udder was controlled, but teats were generally not dipped. Milk samples were obtained with a milk separator, which is also routinely used for test day recording to obtain a representative sample of the total milk of each individual cow.

Both farms practice concentrate-free feeding, cows are grazed in summer and fed with hay, grass and grass-clover silage (no maize silage) in winter. Health problems are primarily treated homoeopathically.

The farmer on farm 1 practices mother bonded calf rearing for more than 11 years, while on farm 2 the system was established for the trial and continued afterwards.

Calves born during the study period (farm 1: first trial: 20.10.2018 – 08.07.2019, second trial: 21.10.2019 – 03.06.2020, and farm 2: 25.08.2019 – 21.05.2020) were distributed according to a randomised block design: two successively born calves (male or female) of the same sex and from mothers of the same parity group (primi- or multiparous) within one farm were randomly distributed to teat bucket feeding or mother suckling. Twins of different sexes were excluded from the study.

All calves were kept with their mothers in a separate barn during the colostrum period for approximately 7 days on farm 1, but for only 2 days on farm 2. On farm 2, dam colostrum feeding for BF calves was continued with the bucket until day 7 post partum.

If necessary, calves were assisted the first day to find the teats. According to the farmers this was rarely necessary.

After seven (farm 1) or two (farm 2) days, calves of both feeding treatments were housed together in one group and only separated during the milk feeding events twice daily. Calves had ad libitum access to hay and fresh water. On farm 1 they were fed carrots daily, but received no additional salt or mineral supplementation. On farm 2 they received salt (Alb. Lehmann AG, Gossau, Switzerland) and minerals for calves (UFA 999, CAKE BLOC, Herzogenbuchsee, Switzerland).

On farm 1 cows and calves of the cow contact feeding treatment had part-time contact twice a day directly after milking (from 8.00 to 8.30 a.m. and from 6.50 to 7.20 p.m, respectively) while teat bucket fed calves received warmed bulk tank milk (38 °C) during the same time.

On farm 2 cows and calves had part-time contact twice a day directly after milking (from 7.30 to 8.00 a.m. and from 6.00 to 6.30 p.m.). Bucket calves were fed warmed bulk tank milk (38 °C) earlier, during milking (from 6.30 to 7.30 a.m. and from 5.30 to 6.00 p.m.). MS calves were present in the calf barn at the beginning of the bucket feeding period, but showed no interest in milk feeding. The access to the exercise yard, the meeting point with their mothers, was opened for them while BF calves received milk.

On both farms, bucket fed calves were fixed during feeding events to prevent competition. Teat buckets were removed within approximately 5–10 min after emptying. BF calves remained fixed for approximately 20 min after milk feeding.

After the end of our trials at the age of 16 weeks all calves of both farms were gradually weaned until the age of approximately 20 weeks.

2.3. Sample sizes and data sampling

2.3.1. Sample sizes

On farm 1, 40 cow-calf couples were involved in two trials of 20 couples each. Six calves died (all male, one BF calf and five MS calves). Data of dead calves was excluded when they lived less than 4 weeks (applicable to 4 calves: 1 BF, 3 MS), additionally one dwarf BF calf was excluded from the trial. This reduced observations to a maximum of 35 calves for data on behaviour, clinical scores and medical treatment (n: BF=18, MS=17), and due to one missing value to 34 calves (BF=18, MS=16) for data on weight gain. Milk and blood sampling started later in trial 1 and samples were available for 23 cows (milk, n: without calf contact= 12, with calf contact= 11) and 24 calves (blood, n: BF=13, MS=11), respectively.

On farm 2, 23 calf-cow couples were involved in the study for all traits, of which 12 calves were BF and 11 were MS calves. On farm 2 no calf died.

In total this resulted in sample sizes of 30 BF and 28 MS calves for the traits on behaviour, clinical scores and medical treatments, and was reduced to 27 MS calves for weight gain. Parameters measured in calf blood were available for 25 BF and 22 MS calves and milk samples were obtained from 23 cows without and 21 cows with calf contact.

2.3.2. Body weight and milk intake

Calves were weighed at birth and in week 1, 2, 3, 4, 8, 12 and 16 of life with a scale (farm 1: AGRETO Animal Scale, AGRETO Electronics GmbH, Raabs, Austria; farm 2: Meier-Brakenberg MB WA 100). Daily weight gain (g/d) was calculated for weeks 1–2, 2–3, 3–4, 4–8, 8–12 and 12–16 on both farms, respectively.

To estimate the milk intake of MS calves they were weekly weighed before and after drinking in the morning. On farm 1 bucket fed calves drank 1000 kg milk within 16 weeks following the nutrition scheme shown in Table 1, while mother fed calves drank around 940 kg within 16 weeks. On farm 2 bucket fed calves drank approximately 888 kg milk compared to 977 kg in mother suckling calves until the age of 16 weeks (Table 1).

2.3.3. Behaviour traits

We counted the number of cross-sucking events (oral manipulation of pen mates) and the number of oral manipulations of objects during three complete hours (from 1.30 to 4.30 p.m.) through continuous behaviour sampling once weekly. The observation time slot was intentionally chosen to reduce any immediate influence of previous feeding on the observed behaviour, as oral manipulations might occur more often directly after drinking. The manipulation had to be performed for at least 5 s to be counted. After an interruption of at least 5 s, the re-performed behaviour was counted as a new event.

After continuous behaviour sampling, we assessed the avoidance distance in all calves present in the pen every two weeks. The assessor approached calves in random order from a distance of around 3 m, holding the right arm overhand in an angle of approximately 45° in front of the body (Waiblinger et al., 2003) at a speed of one step of around 50–60 cm per second, until the animal withdrew the head or tolerated touching (Windschnurer et al., 2008). The distance between the animal's muzzle and the palm of the assessor's hand was estimated in steps of 10 cm.

2.3.4. Clincial assessment

Calves were scored on vitality, body condition, and indicators for diarrhoea and respiratory problems according to the scheme shown in Table 2, adapted from assessment schemes of Aly et al. (2014) and Buczinski et al. (2018). The clinical scoring took place after the behavioural observations for every animal that was sampled that day, respectively.

2.3.5. Observer training and inter-observer reliability assessment

For assessment of inter-observer reliability regarding behaviour of calves a set of 26 videos (duration between 0:16 and 8:26 min:sec) with 4–7 calves per pen (in total 157 datasets on calf level) originating from

Table 1
Estimated milk intake (kg/ day) in bucket fed (BF) and mother suckling (MS) calves in the first 16 weeks of life by farm.

Variable	Week of life	Farm 1		Farm 2	
		BF (n = 18)	MS (n = 16)	BF (n = 12)	MS (n = 11)
Estimated milk intake (kg/d)[a]	1	6.0	6.0	6.7	6.0
	2	7.0	7.0	6.9	7.4
	3	8.7	8.4	7.3	7.2
	4	8.7	8.4	7.6	8.3
	5	8.7	8.4	7.9	8.4
	6	8.7	8.4	7.9	8.4
	7	8.7	7.7	7.9	8.4
	8	9.1	7.8	7.9	8.4
	9	9.1	8.5	8.2	9.3
	10	9.1	9.2	8.2	9.3
	11	9.8	8.3	8.2	9.3
	12	9.8	8.9	8.2	9.3
	13	9.8	9.9	8.5	10
	14	9.8	8.9	8.5	10
	15	10.0	9.6	8.5	10
	16	10.0	8.9	8.5	10
Total milk amount (kg)	1–16	1000	940	888	977

[a] Farm 1: milk intake estimated for weeks 1 and 2; weekly weighing of animals started in week 3; for mother suckling calves weighing was realised before and after drinking, Farm 2: Milk intake for mother suckling calves estimated through weighing in weeks 1, 2, 3, 4, 8, 12, 16; milk intake for weeks in between were estimated by averaging the neighbouring weeks.

Table 2
Clinical scoring scheme.

Category	Parameter	Scores and definitions
Vitality	vitality, general condition	1. lively, agile, vital, reactive, attentive calf 2. little responding or downer calf; and/or hunched back or clearly drooping ears
Body condition	condition	1. good to very good: ribs and spinous processes not visible, prominent bones well rounded 2. moderate: ribs and spinous processes visible and edgy hip bones runt: sharp ribs and striking spinous processes, weak musculature: long back muscle behind shoulder weakly developed, additionally clearly visible tail base without fat cover, weakly developed muscles at the hindquarter
	hair coat	1. even, glossy 2. scrubby and dull - at least half of the thorax
Diarrhoea indicator	diarrhoea, cleanliness	1. clean or only an area not bigger than one palm of a human's hand is dirty around tail 2. manure plaques around tail amounting to at least the size of the palm of a hand (fresh or dry soiling)
Respiratory problems	coughing	1. no 2. single or repeated cough
	nasal discharge	1. no nasal discharge 2. clear effluent dripping from nose or any opaque or purulent discharge
	ocular discharge	1. no ocular discharge 2. clearly visible wet or dry flow from the eye of at least fingers' breadth and/or any crust (at least 0.5 cm maximal length = ½ fingers' breadth)
	breathing	1. normal 2. hampered, forced breathing

five farms was used. A Pearson's correlation coefficient of $r \geq 0.7$ between each observer and the standard was defined as mandatory prior to admission as an observer in the trials. All seven observers involved in the present study reached an r value > 0.7. The average r values were 0.88 ($\pm$ 0.08 SD) and 0.94 ($\pm$ 0.06 SD) for observation of manipulations of objects and for cross-sucking (manipulation of other calves), respectively.

For clinical scoring the consistency between each observer and the standard was tested using at least 30 pictures of calves plus for most of the assessors additional live-observations on 11 calves. The inter-observer reliability for clinical scoring was calculated as Prevalence Adjusted Bias Adjusted Kappa (PABAK) value in Excel and required to be $\geq$ 0.4 between each assessor and the standard. The nine observers involved in the clinical scoring of animals reached a PABAK of > 0.4 for all traits. The average PABAK was 0.71 ($\pm$ 0.27 SD).

2.3.6. Recording of medical treatments

Health state and medical treatments were documented daily by the farmer. Documented treatment reasons were intestinal infections (diarrhoea), respiratory infections, umbilical hernia, locomotion problems, ear infections, reduced vitality and elevated body temperature. Medical treatment data was aggregated at animal level and counted as number of medical treatment cycles. Medical treatments of up to 7 consecutive days for the same reason were counted as one medical treatment cycle (i.e. 1–7 treatment days= 1 cycle, 8–14 treatment days= 2 cycles and so on). If the medical treatment was interrupted for at least seven days, a subsequent medical treatment was counted as a new medical treatment cycle. Administration of analgetics after routine zootechnical procedures (dehorning and / or castration, both only practiced on farm 2) was not counted.

2.3.7. Blood sampling and analysis

Within 48 h post partum a blood sample of 10 ml was taken from the Vena jugularis externa after shaving and disinfection with 70% alcoholic solution using a sterile V2A cannula (Braun-Melsungen, Sterican 1,3 mm). Thereafter, blood sampling of 20 ml of calf blood (divided into 1 ×10 ml EDTA tube and 1 ×10 ml whole blood without anticoagulant) was conducted weekly during the first 4 weeks of calves's life (four sample events) and monthly afterwards (three sample events) until the age of four months.

The blood sample without anticoagulant was stored at room temperature for at least 1 h until the blood coagulated and then spun in a centrifuge (Heareus Multifuge 1 S Centrifuge) at 1300 G for 15 min to obtain serum.

The quality of colostrum supplementation was determined within 48 h post partum by measuring total protein content (g/L) in calf blood serum with a portable refractometer (Euromex, Arnhem, The Netherlands). Thereafter, total protein was determined weekly during the first 4 weeks of calves' lifes (week of life 1, 2, 3, 4) and monthly afterwards until the age of four months (week of life 8, 12, 16).

Immunoglobulin G content (IgG, in mg/dL) of calf blood serum was analysed in the VetAgro Sup laboratory (Marcy l'Etoile, France) using a radial immunodiffusion method (Bovine IgG1 Test from IDBiotech, Issoire, France) to assess their immunity status in week of life 1, 3, 8, 12 and 16, respectively.

We determined glucose content (mg/dL) in fresh uncoagulated blood by using test strips and a quick test analyser (ACCU-CHECK Guide, Roche Diabetes Care Deutschland GmbH, Mannheim, Germany) and lactate content (mmol/L) using a hand-held lactate analyser (Lactate Scout, EFK Diagnostics GmbH, Mannheim, Germany) in week of life 1, 2, 3, 4, 8, 12 and 16.

Packed cell volume (PCV in %) also known as haematocrit, was determined in EDTA conserved blood samples for week of life 1, 2, 3, 4, 8, 12 and 16. Therefore, three capillary tubes per sample were filled with EDTA blood and centrifugated for 5 min at 10'000 rotations/min (Heraeus Pico17 Centrifuge). The PCV was then calculated as the ratio of the column of packed erythrocytes to the total length of the sample in the capillary tube, measured with a graphic reading device (Micro Haematocrit, Thermo Scientific™ 7600-0938). The measurement was performed within 10 min after centrifugation in order to ensure a thorough separation of the layers.

2.3.8. Cow milk sampling and milk analysis

Milk samples were taken directly after calving (colostrum samples) and in week 3, 8, 12 and 16 post partum and frozen at − 18 °C directly after milking. They were transported frozen to FiBL (Research Institute of Organic Agriculture, Frick, Switzerland) and after trials were finished, they were sent to INRAE (Institut national de recherche pour l'agriculture, l'alimentation et l'environnement, Clermont Ferrand, France) within 48 h and without interruption of the cold chain.

All milk samples were analysed in the Agrolabs' laboratory (Aurillac, France) applying an enzyme-linked immunosorbent assay (ELISA) to determine lactoferrin content (mg/L) and a radial immunodiffusion method (Bovine IgG1 Test from IDBiotech, Issoire, France) to determine immunoglobulin G content (mg/L).

2.4. Statistical analysis

We used linear mixed-effects models and generalised linear mixed-effects models in the packages "lme4" (version 1.1-26, Bates et al., 2015) and "lmerTest" (version 3.1-1, Kuznetsova et al., 2017), and generalised linear models in R version 3.6.3 (2020-02-29, R Core Team, 2020) to analyse the effect of feeding treatment on our dependent traits from behaviour observations and samplings described above. The initial models contained feeding treatment (levels: mother suckling= MS or bucket feeding= BF), parity of the mother (levels: primiparous or multiparous), sex of the calf (levels: male or female), time of observation (levels: 1, 2, 3, 4, 8, 12 or 16 weeks of age for weight gain, clinical scores and blood parameters, age in weeks as numeric covariate for behaviour traits, and 3, 8, 12, or 16 weeks post partum for IgG in serum and milk, and lactoferrin in milk), farm (levels: farm 1 or farm 2), and calving season (levels: season 1 from August to November or season 2 from December to March) as fixed factors. In models for total protein content the measurement obtained within 48 h after birth was used as a covariate. In models on immunoglobulin G content in serum of calves we used the measurement during the first week as a covariate. We also examined interactions between the explaining variables in the initial models. Calving year (levels: 2018, 2019 or 2020) and in case of repeated measurements also animal were used as random effects.

Continuously scaled dependent variables (i.e. average daily weight gain, avoidance distance, blood parameters, IgG and lactoferrin in milk) were analysed using linear mixed-effects models (function "lmer" in the "lme4" package).

Dependent variables on count scale (i.e. cross-sucking and oral manipulation of objects) were fit with generalised linear mixed-effects models assuming negative binomial distribution (function "glmer.nb" in the "lme4" package), which had a better fit according to the Akaike information criterion value compared to Poisson distribution.

Binary coded dependent variables (health scores) were analysed as generalised linear mixed-effects models (function glmer" in the "lme 4" package) setting family to "binomial" and using the optimizer "bobyqa".

For dependent traits with data aggregated at animal level (i.e. medical treatment data) we used generalised linear models with Poisson distribution with a logarithmic link function (function "glm" in the "stats" package) including the same explaining variables as described above, apart from time of observation.

Final models were obtained through backwards selection procedures with the function "step" (package "stats", R Core Team, 2020) for continuous scaled dependent variables and medical treatment cycles. For dependent variables on count data level we compared models with the function "anova" and chose the model containing all statistically significant effects and interactions while reducing complexity. Fixed effects and interactions were removed if they were not significant according to the analysis of deviance (Type II Wald chi-squared test) performed in the "car" package (version 3.0-6, Fox and Weisberg, 2019).

Feeding treatment was always kept as fixed effect and in case of repeated measurements at least animal was kept as random effect. For the following dependent variables the random effect calving year was removed from the final model obtained through model selection procedures: vitality, body condition score, cough, ocular discharge, total protein, immunoglobulin G content in milk, lactoferrin content.

The differences between least square means of the fixed effects or in case of relevance of the interactions were verified by Tukey post hoc tests using the "emmeans" package (version 1.4.5, Lenth, 2020) for the respective final model. Apart from binary coded health scores for which we report odd ratios which are back-transformed from the logit scale in "emmeans", we present least square means and standard errors (LSM ± SE). Post hoc tests for non-continuous scaled variables (binary or count data) were performed on the log odds scale in "emmeans", and results back-transformed to the original scale.

Model assumptions were tested by visually inspecting model residuals for deviance from normality (QQ-plots) and homogeneity of variance (plotting fitted vs. residual values). Multicollinearity was assessed by calculating the variance inflation factor.

Statistical significance was assumed at $P < 0.05$.

3. Results

3.1. Average daily weight gain

The final model for average daily weight gain obtained after backwards selection included birth weight as covariate, the interaction of time of observation and feeding treatment and farm as fixed effects, and calf and calving year as random effects.

Average daily weight gain was comparable between feeding treatments, except for the first observation point in time, where MS calves were heavier compared to BF calves (Table 3).

Moreover, we observed significantly higher average daily weight gain on farm 2 compared to farm 1 (LSM ± SE: 863 ± 75 g/d (n = 23 animals, 157 observations) vs. 691 ± 71 g/d (n = 34 animals, 211 observations), $P = 0.0004$).

3.2. Behavioural traits

3.2.1. Cross-sucking and oral manipulations of objects

Fixed effects included in final models for cross-sucking and oral manipulations of objects are shown in Table 4.

Cross-sucking was consistently more frequent in BF calves compared to MS calves (Table 4) on both farms. By contrast, oral manipulations of objects did not differ between feeding treatments (Table 4).

Cross-sucking decreased with age ($P = 0.0124$) and was significantly higher on farm 1 compared to farm 2 (farm 1 vs. farm 2 = LSM ± SE: 1.05 ± 0.16 (n = 35 animals, 412 observations) vs. 0.41 ± 0.07 (n = 23 animals, 350 observations), $P < 0.0001$).

Number of oral manipulations of objects did not differ significantly between calves born in different calving seasons until day 65 of age ($P > 0.05$). From day 66 of life onwards calves born in season 2 (December – March) performed significantly fewer oral manipulations of objects compared to calves born in season 1 (August – November) (Season 1 vs. Season 2: LSM ± SE: 2.98 ± 0.47 (n = 23 animals, 271 observations) vs. 2.35 ± 0.34 (n = 35 animals, 490 observations), $P = 0.038$).

3.2.2. Avoidance distance

The fixed effects of the final model on avoidance distance are shown in Table 4. Average avoidance distance did not statistically differ in calves of different feeding treatments (Table 4). It significantly decreased with age ($P < 0.0001$). Moreover, the interaction between parity of the mother and farm showed a significantly smaller avoidance distance in calves from primiparous cows on farm 2 (primiparous vs. multiparous= LSM ± SE: 0.056 ± 0.260 m (n = 5 animals, 38 observations) vs. 0.465 ± 0.215 m (n = 18 animals, 140 observations), $P = 0.042$).

3.3. Clincial scoring

Results on clinical scores regarding vitality, body condition traits and indicators for diarrhoea and respiratory disorders assessed according to the scheme in Table 2 are shown in Table 5. We did not find statistically significant differences between feeding treatments for any of the clinical scores. Odd ratios of all fixed factors of the respective final models, which significantly contributed to explain variability of clinical scores, are shown in Table 5 as well. For example calves born in calving season 1 showed significantly lower incidences of bad hair condition, less signs of diarrhoea and ocular discharge than those born in calving season 2. Signs of diarrhoea were lowest in weeks 12 and 16, and highest in week 3. By contrast, incidences of nasal and ocular discharge both were lowest in weeks 1 and 2 and highest in weeks 12 and 16. Respective least square means for the time effects are shown as footnotes in Table 5. For the dependent variable forced breathing incidences were very low and models failed to converge. Ocular discharge, dirt around the tail (used as indicator for diarrhoea) and nasal discharge were the most frequently detected health problem indicators under both feeding treatments. Odd ratios between BF and MS calves ranged from 0.74 to 2.39 with high standard errors and were highest for bad body condition (LSM ± SE: 2.39 ± 2.33), followed by impaired vitality (LSM ± SE: 1.59 ± 1.68).

3.4. Medical treatments

Fixed effects remaining in final models on medical treatment cycles

Table 3
Average daily weight gain (g/d) in dairy calves reared by bucket feeding (BF) versus mother suckling (MS) twice a day by feeding treatment and time of observation as least square means (LSM) ± standard error (SE).

Variable	Time of observation	Feeding treatment (Treat)						P
		BF ($n_{animals}$ = 30)			MS ($n_{animals}$ = 27)			
		LSM ± SE	CI	n_{obs}	LSM ± SE	CI	n_{obs}	Treat x Time
Average daily weight gain (g/d)	1	358^{b} ± 98	88–628	23	827^{a} ± 105	551–1104	20	< 0.0001
	2	628 ± 96	358–898	26	667 ± 101	394–941	23	0.701
	3	597 ± 97	326–869	27	661 ± 94	390–931	26	0.521
	4	645 ± 94	384–925	29	713 ± 97	442–985	27	0.547
	8	754 ± 97	483–1025	30	771 ± 93	500–1042	27	0.863
	12	907 ± 93	636–1178	30	1049 ± 97	777–1320	26	0.143
	16	1139 ± 93	868–1410	30	1156 ± 100	884–1428	24	0.865

$n_{anmials}$ = number of animals, CI = confidence interval, n_{obs} = number of observations, different superscripts between LSM indicate significant differences according to post hoc tests at P < 0.05.

Table 4
Least square means (LSM) and standard errors (SE) of average number of cross-sucking (oral manipulation of pen mates, CrossSuck) and oral manipulations of objects (ManObj) during weekly repeated direct continuous behaviour sampling of three hours (13.30–16.30 p.m.), average avoidance distance (AVD) of calves assessed forthnightly, total protein content (TotalProt), glucose, and packed cell volume (PCV) measured in calf blood or serum at week 1, 2, 3, 4, 8, 12 and 16 of life, immunoglobulin G (IgG) measured in week 3, 8, 12 and 16 of life, and total number of medical treatment cycles (numTotalTreat) and number of medical treatment cycles with homoepathic remedies or dietary products (numHomTreat) aggregated at animal level for the first four months of life in calves reared with bucket feeding (BF) or mother suckling (MS) twice a day.

Variable	Feeding treatment (Treat)						P-value[1]						Interactions and covariates (cov) with P-value
	BF			MS									
	LSM ± SE	$n_{animals}$	n_{obs}	LSM ± SE	$n_{animals}$	n_{obs}	Treat	Time	Sex	Farm	Parity	Calving season	
CrossSuck (n)	0.83^{a} ± 0.13	30	400	0.53^{b} ± 0.09	28	362	0.003	0.0124		< 0.0001			
ManObj (n)	2.43 ± 0.35	30	399	2.74 ± 0.40	28	362	0.171	int				int	Time*Calving season < 0.0001
AVD (m)	0.480 ± 0.082	30	203	0.474 ± 0.085	28	178	0.954						
TotalProt (g/L)	58.7 ± 0.6	30	176	59.0 ± 0.6	28	122	0.753	int	int	int	int	int	cov: total protein 48 h 0.006 Time*Parity 0.021 Farm*Parity 0.037 Calving season*Parity 0.022 Calving season*Sex 0.018
IgG (mg/L)	12.9 ± 0.8	25	91	12.4 ± 0.8	22	84	0.595	0.0004					cov: IgG in week 1 0.022
Glucose (mg/dL)	109 ± 3	25	156	114 ± 3	22	135	0.030	< 0.0001		int	int		Parity*Farm 0.005
PCV (%)	29.2 ± 0.9	25	160	32.7 ± 1.0	22	137	0.0005	int	int	int	int	int	Sex*calving season 0.045 Parity*Time 0.012 Time*Farm < 0.0001
numTotalTreat	1.91 ± 0.28	30		2.15 ± 0.30	28		0.500	/		int	int		Parity*Farm 0.043
numHomTreat	1.39 ± 0.25	30		1.67 ± 0.26	28		0.436	/		int	int		Parity*Farm 0.044

n_{anim} = number of animals, n_{obs} = number of observations, int = involved in interaction, LSM with different superscripts indicate significant differences according to post hoc tests at P < 0.05, [1]P-values from the analysis of deviance (Type II Wald chi-squared test), Time: age in days for CrossSuck, ManObj, and AVD; week 1, 2, 3, 4, 8, 12, 16 for TotalProt, Glucose, and PCV; week 3, 8, 12, 16 for IgG; not applicable to medical treatment cycles as data was aggregated at animal level from day of birth until end of observation at 16 weeks of age, Sex: male and female, Farm: farm1 and farm2, parity: primiparous and multiparous, calving season: season 1 (Aug-Nov) and season 2 (Dec-March).

are shown in Table 4. The average number of medical treatment cycles, total and alternative, did not statistically differ between feeding treatments (Table 4). The interaction between farm and parity of the mother revealed, that number of total medical treatment cycles was significantly higher on farm 1 in calves from multiparous cows (P < 0.0001), but did not differ between farms in calves from primparous cows (P = 0.468). While calves from multiparous cows on farm 2 had less than one medical treatment cycle on average (LSM ± SE: 0.99 ± 0.23, n = 18), these calves had close to three medical treatment cycles on farm 1 (LSM ± SE: 2.96 ± 0.38, n = 21). On farm 2 medical treatment cycles were exclusively homoeopathic, while animals on farm 1 had other medical treatments than with homoeopathy. Average number of alternative medical treatment cycles differed significantly between farms in calves from multiparous cows, again, (farm 1 vs. farm 2: LSM ± SE: 1.88 ± 0.30 (n = 21) vs. 0.92 ± 0.23 (n = 18), P = 0.014), but not so in calves from primiparous cows (P = 0.524).

3.5. *Blood analysis*

With the exception of the model for lactate, all fixed effects, covariates, and interactions of the final models for the blood parameters presented in this section are shown in Table 4.

Table 5
Descriptive proportions of clinical scores in calves by feeding treatment (bucket feeding = BF, mother suckling = MS) and odd ratios derived from post hoc tests, standard errors in brackets (SE).

Variable	Feeding treatment (Treat)				Parity of the mother (Parity)		Sex		Calving season (Season)			Farm	Time
	Descriptive proportions		Odd ratio BF/MS		Odd ratio multiparous/ primparous		Odd ratio female/ male		Odd ratio season1/ season2		Odd ratio farm 1/ farm 2		
	BF	MS											
$n_{animals}$	30	28	30/28		39/19		31/27		23/35		35/23		
n_{obs}	195	184	195/ 184	P_{Treat}	261/118	P_{Parity}	207/172	P_{sex}	148/231	P_{Season}	218/161	P_{Farm}	$P^{\dagger}_{Time}$
Impaired vitality	5.1	3.3	1.59 (SE: 1.68)	0.659									
Bad body condition	4.6	2.2	2.39 (SE: 2.33)	0.374									
Bad hair coat condition	3.6	4.9	0.62 (SE: 0.34)	0.384	0.19 (SE: 0.11)	0.005	5.83 (SE: 4.11)	0.013	0.19 (SE: 0.13)	0.012			
Dirt around the tail (diarrhoea indicator)	28.7	28.8	1.07 (SE: 0.34)	0.831					0.29 (SE: 0.00)	< 0.0001			< 0.0001[a]
Cough	14.4	15.2	0.94 (SE: 0.32)	0.852							0.23 (SE:0.08)	< 0.0001	0.028[b]
Nasal discharge	26.7	32.1	0.74 (SE: 0.24)	0.352									< 0.0001[c]
Ocular discharge	49.2	45.1	1.38 (SE: 0.32)	0.168					0.28 (SE: 0.07)	< 0.0001			< 0.0001[d]
Forced breathing*	0	0.005	/										

n_{animal} = number of calves, n_{obs} = number of observations, * one missing observation reduced n_{obs} to 183 for this trait, / = models did not converge, P values of fixed effects derived from the post hoc tests performed on the log odds ratio scale in emmeans, †: P-value from the analysis of deviance (Type II Wald chi-squared test), Time effects were back-transformed from the log scale in emmeans, different superscripts between least square means (LSM) indicate significant differences according to post hoc tests at $P < 0.05$:

[a] LSM ± SE: **1:** 22.1^{abc} ± 5.8, **2:** 37.1^{cd} ± 7.6, **3:** 51.9^{d} ± 7.8, **4:** 35.7^{bcd} ± 7.3, **8:** 11.1^{ab} ± 4.3, **12:** 9.8^{a} ± 3.8, **16:** 4.8^{a} ± 2.5.
[b] LSM ± SE: **1:** 1.4^{a} ± 1.5, **2:** 5.2^{ab} ± 3.1, **3:** 15.9^{ab} ± 5.2, **4:** 14.6^{ab} ± 5.1, **8:**. 26.7^{b} ± 6.8, **12:** 14.7^{ab} ± 5.0, **16:** 16.6^{ab} ± 5.3.
[c] LSM ± SE: **1:** 7.9^{a} ± 4.6, **2:** 7.7^{a} ± 4.7, **3:** 21.0^{ab} ± 9.5, **4:** 16.7^{ab} ± 8.1, **8:** 25.8^{abc} ± 11.1, **12:** 49.4^{c} ± 13.6, **16:** 34.9^{bc} ± 12.4.
[d] LSM ± SE: **1:** 10.8^{a} ± 3.8, **2:** 29.7^{ab} ± 6.5, **3:** 41.0^{bc} ± 6.9, **4:** 40.2^{bc} ± 7.0, **8:** 57.4^{bc} ± 7.6, **12:** 67.6^{c} ± 6.7, **16:** 62.6^{c} ± 6.9.

3.5.1. *Total protein*

Passive immune transfer failure, defined as a value < 50 g/L of total protein in calf serum (Buczinski and Vandeweerd, 2016; Godden et al., 2019), occurred in four BF and five MS out of the 22 calves sampled within 48 h post partum in each feeding treatment group, respectively. This corresponded to a failure rate of 18.2% and 22.7% in BF and MS calves, respectively.

No effect of feeding treatment was found for average total protein content (Table 4). Although interactions were statistically significant according to the analysis of deviance (Type II Wald chi-squared test) (Table 4), post hoc tests revealed no significant differences between least square means at the level of $P < 0.05$ for all combinations of time of observation*parity, farm*parity, and calving season*parity, respectively. Female calves born in calving season 2 showed a higher average total protein value compared to males (LSM ± SE: female: 59.8 ± 0.7 (n animals= 16, n observation= 104) vs. male: 56.3 ± 1.0 (n animals= 11, n observations= 69), P = 0.002), but this difference between sexes was not significant in calves born in calving season 1 (P = 0.911).

3.5.2. *Immunoglobulin G*

Immunoglobulin G content (mg/L) in calf serum did not differ between feeding treatments (Table 4), but increased with time (LSM ± SE: time 3 = 10.6^{a} ± 0.9 (n = 44), time 8 = 12.3^{ab} ± 0.9 (n = 41), time 12 = 12.9^{ab} ± 0.9 (n = 44), time 16 = 14.8^{b} ± 0.9 (n = 46), where different superscripts indicate significant differences according to post hoc tests at $P < 0.05$).

3.5.3. *Glucose*

The mean blood glucose content (mg/L) was significantly higher in MS compared to BF calves (Table 4). It was higher in calves of multiparous cows on farm 2 (LSM ± SE: multiparous: 120 ± 2.7 (n animals= 18, n observations= 108) vs. primiparous: 105 ± 4.1 (n animals= 5, n observations= 32), P = 0.0008), but did not differ between calves from cows of different parity status on farm 1 (P = 0.883). Moreover, average glucose level differed by time of observation, it was highest in week 1 and lowest in weeks 12 and 16, respectively (LSM ± SE: time 1 = 119^{c} ± 3.2 (n = 26), time 2 = 111^{abc} ± 2.8 (n = 46), time 3 = 115^{bc} ± 2.9 (n = 45), time 4 = 114^{abc} ± 2.9 (n = 45), time 8 = 108^{ab} ± 3.0 (n = 39), time 12 = 108^{a} ± 2.9 (n = 45), time 16 = 108^{a} ± 2.9 (n = 45), where different superscripts indicate significant differences according to post hoc tests at $P < 0.05$).

3.5.4. *Lactate*

The final model on lactate content included sex of the calf and the interactions feeding treatment*farm and time of observation*farm as fixed factors, and calving year and calf as random factors. While lactate content was significantly higher in blood of BF calves on farm 1 (LSM ± SE: BF: 1.29 ± 0.09 (n animals= 13, n observations= 69) vs. MS: 1.05 ± 0.10 (n animals= 11, n observations= 56), P = 0.016), it did not significantly differ between feeding treatments on farm 2 (LSM ± SE: BF: 0.73 ± 0.09 (n animals= 12, n observations= 72) vs. MS: 0.86 ± 0.09 (n animals= 11, n observations= 60), P = 0.147). It was higher in female compared to male calves (LSM ± SE: female: 1.07 ± 0.08 (n animals=

27, n observations= 142) vs. male: 0.90 ± 0.08 (n animals= 20, n observations= 115), P = 0.016). The interaction of time of observation and farm demonstrated constant values for lactate on farm 1 over time (LSM ranged from 1.00 to 1.35), while they were significantly lower in week 16 compared to week 1, 2,3, 4, and 8 on farm 2 (LSM ± SE: time 1 = 1.10^{a} ± 0.16 (n = 13), time 2 = 0.87^{a} ± 0.13 (n = 22), time 3 = 0.88^{a} ± 0.12 (n = 23), time 4 = 0.84^{a} ± 0.13 (n = 22), time 8 = 0.96^{a} ± 0.14 (n = 18), time 12 = 0.64^{ab} ± 0.14 (n = 16), time 16 = 0.29^{b} ± 0.14 (n = 18), where different superscripts indicate significant differences according to post hoc tests at $P < 0.05$).

3.5.5. PCV

Packed cell volume was significantly higher in MS compared to BF calves (Table 4). It was also higher in female compared to male calves born in calving season 2 (LSM ± SE: female 32.1 ± 1.6 (n animals= 16, n observations=105) vs. male 27.9 ± 1.2 (n animals= 11, n observations= 70), P = 0.018), but did not differ by sex in calves born in calving season 1 (P = 0.609).

Average PCV values in calves from multiparous cows, were highest in the first two weeks, then decreased until the lowest value in week 8, and were back at a high level in week 16 (LSM ± SE: time 1 = 33.6^{c} ± 1.3 (n = 19), time 2 = 33.3^{c} ± 1.1 (n = 33), time 3 = 32.2^{bc} ± 1.1 (n = 33), time 4 = 32.2^{bc} ± 1.1 (n = 32), time 8 = 28.6^{a} ± 1.1 (n = 31), time 12 = 29.8^{ab} ± 1.1 (n = 16), time 16 = 32.8^{c} ± 1.1 (n = 33), where different superscripts indicate significant differences according to post hoc tests at $P < 0.05$). In calves born by primiparous cows, however, no statistically significant difference was found at any time of observation (LSM ranged from 27.5 to 32.9).

Finally, PCV values changed differently over time by farm, but no clear pattern was recognisable (results not shown).

3.6. Immunoglobulin and lactoferrin content in cows' milk

Immunoglobulin G content (mg/L) in cows' milk did not differ between feeding treatments (Table 6), it was higher in cows calving in season 1 and considerably decreased from week 3 onwards (Table 6, Fig. 1a). Although variability of lactoferrin content (mg/L) in cows' milk was higher in cows with calf contact (Fig. 1b), least square means did not differ between feeding treatments (Table 6), nor did they vary with time (Fig. 1b).

4. Discussion

4.1. Average daily weight gain

The comparable average weight gains in both feeding treatments, show that we successfully managed to avoid an effect of different milk quantities in feeding treatments. We lack explanation for the pronounced difference found in favour of MS calves in week 1, especially for farm 1, where permanent access to the dam was practiced for calves of both feeding treatments until 7 days post partum. The higher weight gains on farm 2 could be explained by a better health situation as reflected by considerably fewer medical treatment cycles found on this farm.

4.2. Behavioural traits

4.2.1. Cross-sucking and oral manipulations of objects

We observed less cross-sucking in MS calves compared to BF pen mates. These findings are in line with other studies reporting less cross-sucking in mother-bonded calves compared to calves reared artificially (e.g. Fröberg et al., 2008; reviewed by Kälber and Barth, 2014; reviewed by Johnsen et al., 2016; reviewed by Meagher et al., 2019; Roth et al., 2009). Some studies report less sucking events due to higher milk allowance (e.g. Jung and Lidfors, 2001), but this cannot be the explanation in our study, as milk levels were comparable on purpose. Therefore, our findings indicate that mother contact even in a restrictive form seems to better satisfy the natural sucking needs of calves than teat bucket feeding does.

Contrary to our hypothesis, we could not find differences regarding the number of oral manipulations of objects in favour of MS calves. This finding is not in line with studies reporting less frequent licking of objects by calves in CCC systems (with part-time suckling: Fröberg et al., 2008; four days contact: Krohn et al., 1999). While another study reported more licking of objects in dam suckling calves with half-day contact compared to artificially reared calves (Veissier et al., 2013).

4.2.2. Avoidance distance

Because CCC systems are partly associated with the risk of feral calves (Krohn et al., 1999), avoidance distance tests were carried out. The fact that there was no difference between the feeding treatments indicates that part-time contact with the mother twice daily studied here, did not influence the attitude towards humans compared to bucket feeding. By contrast, Waiblinger et al. (2020) reported that artificially fed calves showed lower avoidance distance compared to calves with dam contact. The animal-human contact in the study of Waiblinger et al. (2020) was less frequent compared to our study: artificially reared calves were fed by automatic feeders from day 5 post partum onwards and calves of the dam group had permanent access to the dam. By contrast, in our study daily handling of calves of both feeding treatments for the feeding events and frequent weighing led to more interactions between humans and animals. Additionally, the contact to the dam was limited to twice daily in our study. Krohn et al. (1999) reported that female dairy calves with whole-day cow contact for only 4 days post partum were more difficult to approach as 15–18 months old heifers compared to heifers without mother contact or shorter mother contact directly after birth. The decreasing avoidance distance with age that we found, is in line with results reported by Waiblinger et al. (2020), although from a different study design (see outline above). It can be explained by a habituation effect towards humans through daily routine

Table 6
Content of immunoglobulin G (lgG) and lactoferrin in cow milk from cows with or without calf contact averaged over measurements at week 3, 8, 12 and 16 post partum as least square means (LSM), standard error (SE) and confidence intervals (CI).

	Milk of cows					Time of sampling								Calving season			
	without calf contact ($n_{animals}$ = 23)		with calf contact ($n_{animals}$ = 21)			3		8		12		16		1 ($n_{animals}$ = 16)		2 ($n_{animals}$ = 17)	
Variable	LSM ± SE	n_{obs}	LSM ± SE	n_{obs}	P	LSM ± SE	n_{obs}	LSM ± SE	n_{obs}	LSM ± SE	n_{obs}	LSM ± SE	n_{obs}	LSM ± SE	n_{obs}	LSM ± SE	n_{obs}
IgG (mg/L)	391 ± 29	82	363 ± 31	83	0.495	477^{a} ± 31	42	351^{b} ± 32	38	341^{b} ± 31	42	339^{b} ± 30	43	427^{a} ± 34	62	327^{b} ± 27	103
Lactoferrin (mg/L)	199 ± 55	82	237 ± 55	83	0.250												

$n_{animals}$ = number of animals, n_{obs} = number of observations, P values of the contact effect derived from the post hoc tests, different superscripts between LSM indicate significant differences according to post hoc tests at $P < 0.05$.

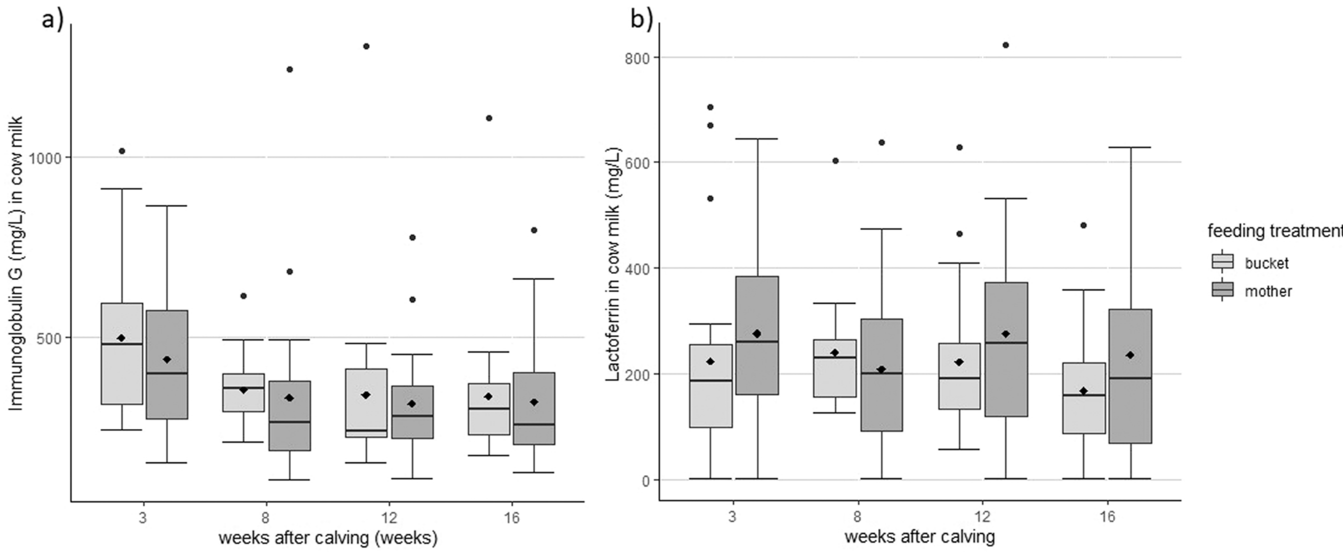

Fig. 1. Content of immunoglobulin G (IgG in mg/L) and lactoferrin (mg/L) in milk of cows with (mother) and without calf contact (bucket) at week 3, 8, 12 and 16 post partum. Lower whisker: minimum, lower end of the box: 25% quartile, line in the box: median, rhombus: mean, upper end of the box: 75% quartile, upper whisker: maximum, dots: outliers.

and also through repeated testing. The smaller avoidance distance found in calves of primiparous cows on farm 2 might result from special care for primiparous cows on this farm during calving. Farm 2 is a family-run farm where four family members take care of the dairy herd. Farm 1, on the other hand, had a higher turnover of personnel and a lower animal to staff ratio in the barn during the trial period, so that no comparable intensity of care can be assumed.

4.3. Clincial assessment and medical treatments

We did not find statistically significant differences between feeding treatments regarding clinical scores or incidences of health problems. In accordance with our findings, no impact of the rearing system on incidences of health problems was reported by Hillmann et al. (2019) in a study involving 39 farms.

In line with most of the studies on diarrhoea in CCC systems (n = 8) reviewed by Beaver et al. (2019) we found no effect of suckling on indicators for diarrhoea. However, we therewith could not confirm the positive effect of suckling found in 6 studies reported in the same review. The occurrence of the most frequent signs of diarrhoea in the third week of life corresponds quite well with the immune gap at this time.

Additionally, the same review could not detect a significant effect of CCC on respiratory risks (Beaver et al., 2019), which is in line with the fact that we did not find differences regarding indicators of respiratory problems between feeding treatments in this study.

The ranking of health problems (ocular discharge, dirt around the tail, and nasal discharge being most frequent in both feeding treatments) is in line with the most common health problems reported in calf rearing (reviewed by Uetake, 2013). The observation that signs of diarrhoea were most prevalent in week 3 of the neonatal period, whereas nasal and ocular discharges were more prevalent later in life, is consistent with temporally different mortality risks due to diarrhoeal and respiratory diseases reported by Uetake (2013).

The pronounced differences in average number of medical treatment cycles between farms found for calves from multiparous cows underline the effect of overall management on animal health, regardless of the rearing system.

4.4. Blood analysis

4.4.1. Passive immune transfer, average total protein and immunoglobulin G

As duration of colostrum feeding did not differ between BF and MS calves no conclusion on passive immune transfer via colostrum for differing feeding treatments can be derived from our study. Nevertheless, the percentages of failed immune transfer observed (BF= 18.2 and MS= 22.7%), which are above the optimum of less than 10% of calves with < 51 g/L total protein (Lorenz, 2021), indicate the need to improve colostrum management in these farms.

In contrast to our findings, Hillmann et al. (2019) found a positive impact of cow contact on total protein content. Least square mean values were within the normal range of total protein content of 50–70 g/L (Baumeister, 2020, p. 136). According to a recent review, calving season might impact colostrum quality: e.g. heat stress in summer might impair transfer of IgG (Godden et al., 2019). This might be part of the explanation for a better performance of female calves in season 2. However, it remains unclear why this could only be observed in female calves.

The numerous interactions in the model on total protein content indicate the high complexity in explaining variance observed for this trait. Further studies with matched pairs in bigger herds might be needed to better elucidate the dynamics impacting this trait.

On the one hand, our findings on IgG content in serum do not match a review reporting that calves with dam contact showed higher rates of IgG absorption and serum IgG concentrations (Weaver et al., 2000), but are in line with the study of Hillmann et al. (2019) which did not identify differences in IgG concentrations in rearing systems with longer cow contact compared to artificially reared calves. The decrease and subsequent rise of IgG concentrations we found with time, conclusively reflect the well known decline of passive immune defence and the further build-up of an active immune defence.

4.4.2. Glucose

Glucose values were in the normal range expected in pre-weaned calves (i.e. 4.4–6.9 mmol/L = 79.3–124.3 mg/ dL according to Baumeister, 2020, p. 136). The slightly, but significantly higher glucose levels in the blood of MS calves might be due to the fact that they ingested the fattier fraction of the milk as they suckled after milking, while BF calves received bulk tank milk of all milked cows, including suckled (i.e. with less fatty milk, as their udders were not emptied completely). The MS calves might have covered a somewhat higher proportion of their energy requirement by fat. As higher glucose levels in the blood are a signal of satiety (Khan et al., 2011), our finding may also be explanatory for the result that MS calves exerted less cross-sucking, as also reported for calves receiving glucose enriched milk (2 g/L) (Egle, 2005).

The decrease of glucose levels we observed with age, is well described in the literature (e.g. McCarthy and Kesler, 1956). It is associated with changes in the energy metabolism from primary supply via glucose as metabolite to energy supply via volatile fatty acids in the mature ruminant (McCarthy and Kesler, 1956).

We could not find literature giving a conclusive explanation as to our findings on higher glucose content in calves from multi- compared to those from primiparous cows on farm 2.

4.4.3. Lactate

The results on lactate levels were contradictory between farms (farm 1: BF > MS, P = 0.016, farm 2: BF ≤ MS, P = 0.147). Apart from the lactate values, which can indicate stress, the avoidance distance was also greater on farm 1 than on farm 2 (numerical results not shown). Calves seemed to be more fearful and stressed on farm 1 compared to farm 2, which was also the subjective impression of assessors. This is also reflected in the higher mean lactate values found on farm 1, which did not change over time. By contrast, calves on farm 2 achieved lower values in week 16.

4.4.4. PCV

Packed cell volume was significantly higher in MS compared to BF calves. Low PCV values can indicate occurrence of iron deficiency anaemia (less erythrocytes), while an elevated PCV value can indicate dehydration (plasma volume decrease). While BF calves were slightly under the norm reference for calves (30–36%, Klee and Hartmann, 2017), this was not the case in MS calves. Although this difference was statistically significant the physiological relevance remains unclear. Further studies might be needed in order to validate our findings, also given the presence of several interactions.

4.5. Immunoglobulin and lactoferrin content in cow's milk

The content of IgG and lactoferrin in milk of cows with our without calf contact did not statistically differ. The decrease of IgG in milk over time is well described in the literature (e.g. Godden et al., 2019). Variability of lactoferrin levels was greater in cows with calf contact than in those without contact. This could refer to a higher reactivity of milk lactoferrin levels because of environmental factors. In particular, a modification of lactoferrin levels in response to bacteriological infections of the cow udder (Chaneton et al., 2008) could be explanatory. However, as udder health was not the focus of the present study, our finding needs further investigation. Although several studies investigated the effect of CCC on cell count (e.g. reviewed by Beaver et al., 2019), fat and protein content on dairy milk (e.g. reviewed by Johnsen et al., 2016), we are not aware of any studies that have investigated the impact of CCC on IgG or lactoferrin in milk and conclude that under the part-time contact conditions of this study no impact of calf contact on average values of these parameters could be observed.

5. Conclusions

As even part-time cow contact twice daily significantly reduced cross-sucking, it can be regarded as a suitable means to satisfy the suckling needs of calves. When fed at comparable milk levels, weight gain did not profit from part-time cow contact alone. Calves with part-time cow contact showed higher glucose content in the blood, which as a signal of satiety might have contributed to reduce cross-sucking in this feeding treatment. By contrast, number of manipulations of object did not differ between feeding treatments. Avoidance distance towards humans did not differ by feeding treatment, indicating that in calves daily handled by humans, feeding with buckets or with part-time daily cow contact does not influence animal-human relationship. Regarding health traits we conclude that part-time access to the mother (twice a day) alone does only have very limited effects, while differences between farms underline the relevance of management. It remains unclear why the average packed cell volume of calves with mother contact was at a normal range, while the values of bucket fed calves was lower. This should be further validated. We conclude that under comparable length of colostrum intake in calves with and without cow contact, part-time cow-calf contact twice daily had no effect on the build-up of the active immune defence during the pre-weaning period until the age of four months. Prolonged contact to the calf did not modulate the investigated content of antimicrobial compounds in dairy milk.

Declaration of Competing Interest

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

Acknowledgements

The authors acknowledge the financial support for the ProYoung-Stock project provided by the Federal Office for Agriculture FOAG as the Swiss transnational funding body (contract number: 627001052) and Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement as the French transnational funding body, being partners of the H2020 ERA-net project, CORE Organic Cofund, and the cofund from the European Commission. The authors acknowledge the financial support from the French government IDEX-ISITE initiative 16-IDEX-0001 (CAP 20-25) and from LiDL Switzerland. We thank the farmers of the two farms where we carried out the study for their commitment and interest in the questions of the study and their professional exchange, their careful data collection and their hospitality during our data collection.We also thank Matthis Walkenhorst for his support in data sampling on farm 1 in the second run of the trial, and Noelle Thüer for her support in calf handling during data sampling on farm 2.

References

Aly, S.S., William, J.L., Deniece, R.W., Lehenbauer, T.W., van Eenennaam, A., Drake, C., Kass, P.H., Farver, T.B., 2014. Agreement between bovine respiratory disease scoring systems for pre-weaned dairy calves. Anim. Health Res. Rev. 15, 148–150. https://doi.org/10.1017/S1466252314000164.

Bar-Peled, U., Robinzon, B., Maltz, E., Tagari, H., Folman, Y., Bruckental, I., Voet, H., Gacitua, H., Lehrer, A.R., 1997. Increased weight gain and effects on production parameters of Holstein heifer calves that were allowed to suckle from birth to six weeks of age. J. Dairy Sci. 80, 2523–2528. https://doi.org/10.3168/jds.S0022-0302(97)76205-2.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Baumeister, C., 2020. Labordiagnostik in der Tierarztpraxis. Ein Handbuch für Tiermedizinische Fachangstellte, third ed. Lehmanns Media, Berlin, Germany.

Beaver, A., Meagher, R.K., von Keyserlingk, M.A.G., Weary, D.M., 2019. Invited review: a systematic review of the effects of early separation on dairy cow and calf health. J. Dairy Sci. 102, 5784–5810. https://doi.org/10.3168/jds.2018-15603.

Buczinski, S., Vandeweerd, J.M., 2016. Diagnostic accuracy of refractometry for assessing bovine colostrum quality: a systematic review and meta-analysis. J. Dairy Sci. 99, 7381–7394. https://doi.org/10.3168/jds.2016-10955.

Buczinski, S., Fecteau, G., Dubuc, J., Francoz, D., 2018. Validation of a clinical scoring system for bovine respiratory disease complex diagnosis in preweaned dairy calves using a Bayesian framework. Prev. Vet. Med. 156, 102–112. https://doi.org/10.1016/j.prevetmed.2018.05.004.

Busch, G., Weary, D.M., Spiller, A., von Keyserlingk, M.A.G., 2017. American and German attitudes towards cow–calf separation on dairy farms. PLoS One 12 (3), e0174013.

Chaneton, L., Tirante, L., Maito, J., Chaves, J., Bussmann, L.E., 2008. Relationship between milk lactoferrin and etiological agent in the mastitic bovine mammary gland. J. Dairy Sci. 91, 1865–1873. https://doi.org/10.3168/jds.2007-0732.

Egle, B., 2005. Verhaltensbeobachtungen zum gegenseitigen Besaugen von Fleckviehkälbern (Ph.D. thesis). 148 pages. ⟨https://sundoc.bibliothek.uni-halle.de/diss-online/05/06H044/prom.pdf⟩ (Accessed 20 December 2021).
Eriksson, H., Fall, N., Priolo, A., Caccamo, M., Michaud, A., Pomies, D., Fuerst-Waltl, B., Weissensteiner, R., Winckler, C., Spengler Neff, A., Bieber, A., Schneider, C., Sakowski, T., Stachelek, M., Ivemeyer, S., Simantke, C., Knierim, U., Alvåsen, K., 2021. Strategies for keeping dairy cows and calves together on European farms. In: European Federation of Animal Science (Ed.), Book of Abstracts of the 72nd Annual Meeting of the European Federation of Animal Science, Davos, Switzerland, 30th August – 3rd September, 2021. Wageningen Academic Publishers, The Netherlands, p. 480. https://doi.org/10.3920/978-90-8686-918-3.
Flower, F.C., Weary, D.M., 2003. The effects of early separation on the dairy cow and calf. Anim. Welf. 12, 339–348.
Fox, J., Weisberg, S., 2019. An {R} Companion to Applied Regression, third ed. Sage, Thousand Oaks, CA (URL: ⟨https://socialsciences.mcmaster.ca/jfox/Books/Companion/⟩).
Fröberg, S., Gratte, E., Svennersten-Sjaunja, K., Olsson, I., Berg, C., Orihuela, A., Galina, C.S., Garcia, B., Lidfors, L., 2008. Effect of suckling ('restricted suckling') on dairy cows' udder health and milk let-down and their calves' weight gain, feed intake and behaviour. Appl. Anim. Behav. Sci. 113, 1–14. https://doi.org/10.1016/j.applanim.2007.12.001.
GEH, 2020. Deutsches Schwarzbuntes Niederungsrind. ⟨http://www.g-e-h.de/index.php/rassebeschreibungen/34-rassekurzbeschreibungen-rinder/70-deutsches-schwarbuntes-niederungsrind⟩ (Accessed 08 October 2020).
Godden, S.M., Lombard, J.E., Woolums, A.R., 2019. Colostrum management for dairy calves. Vet. Clin. Food Anim. 35, 535–556. https://doi.org/10.1016/j.cvfa.2019.07.005.
Hillmann, E., Bruckmaier, R., Buchli, C., 2019. Gesündere Kälber durch kuhgebundene Aufzucht? In: Internationale Gesellschaft für Nutztierhaltung (IGN) (Eds.), Nutztierhaltung im Fokus. Kälberaufzucht- Aspekte verschiedener Nutzungsformen. Informationsbroschüre der IGN e.V. über aktuelle Ergebnisse aus der Forschung zum Wohlbefinden der Tiere, München, Germany, pp. 9–12.
Johnsen, J.F., Zipp, K.A., Kälber, T., de Passillé, A.M., Knierim, U., Barth, K., Mejdall, C. M., 2016. Is rearing calves with the dam a feasible option for dairy farms?—Current and future research. Appl. Anim. Behav. Sci. 181, 1–11. https://doi.org/10.1016/j.applanim.2015.11.011.
Jung, J., Lidfors, L., 2001. Effects of amount of milk, milk flow and access to a rubber teat on cross-sucking and non-nutritive sucking in dairy calves. Appl. Anim. Behav. Sci. 72, 201–213. https://doi.org/10.1016/S0168-1591(01)00110-1.
Kälber, T., Barth, K., 2014. Practical implications of suckling systems for dairy calves in organic production systems- a review. Landbauforsch. Appl. Agric. For. Res. 64, 45–58. https://doi.org/10.3220/LBF_2014_45-58.
Khan, M.A., Weary, D.M., von Keyserlingk, M.A.G., 2011. Invited review: Effects of milk ration on solid feed intake, weaning, and performance in dairy heifers. J. Dairy Sci. 94, 1071–1081. https://doi.org/10.3168/jds.2010-3733.
Klee, W., Hartmann, I., 2017. Klinische Labordiagnostik in der Buiatrik. Download from ⟨http://www.rinderskript.net/skripten/Laborskript.pdf⟩ (Accessed 12 July 2021).
Krohn, C.C., Foldager, J., Mogensen, L., 1999. Long-term effect of colostrum feeding methods on behaviour in female dairy calves. Acta Agric. Scand. 49, 57–64. https://doi.org/10.1080/090647099421540.
Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest package: tests in linear mixed effects models. J. Stat. Softw. 82, 1–26. https://doi.org/10.18637/jss.v082.i13.
Lenth, R., 2020. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.4.5. ⟨https://CRAN.R-project.org/package=emmeans⟩.
Lorenz, I., 2021. Calf health from birth to weaning - an update. Ir. Vet. J. 74, 5. https://doi.org/10.1186/s13620-021-00185-3.
Manzoni, P., Dall'Agnola, A., Tomé, D., Kaufman, D.A., Tavella, E., Pieretto, M., Messina, A., De Luca, D., Bellaiche, M., Mosca, A., Piloquet, H., Simeoni, U., Picaud, J.-C., Del Vecchio, A., 2018. Role of lactoferrin in neonates and infants: an update. Am. J. Perinatol. 35 (06), 561–565. https://doi.org/10.1055/s-0038-1639359.
McCarthy, R.D., Kesler, E.M., 1956. Relation between sge of calf, blood glucose, blood and rumen levels of volatile fatty acids, and in vitro cellulose digestion. J. Dairy Sci. 39, 1280–1287. https://doi.org/10.3168/jds.S0022-0302(56)94846-9.
Meagher, R.K., Beaver, A., Weary, D.M., von Keyserlingk, M.A.G., 2019. Invited review: A systematic review of the effects of prolonged cow-calf contact on behavior, welfare, and productivity. J. Dairy Sci. 102, 5765–5783. https://doi.org/10.3168/jds.2018-16021.
Mendoza, A., Cavestany, D., Roig, G., Ariztia, J., Pereira, C., La Manna, A., Contreras, D. A., Carlos, S., Galina, C.S., 2010. Effect of restricted suckling on milk yield, composition and flow, udder health, and postpartum anoestrus in grazing Holstein cows. Livest. Sci. 127, 60–66. https://doi.org/10.1016/j.livsci.2009.08.006.
de Oliveira, D., Barth, K., Haskell, M.J., Hillmann, E., Jensen, M.B., Johnsen, J.F., Mejdell, C., Waiblinger, S., Ferneborg, S., 2020. Methodology for experimental and observational animal studies in cow-calf contact systems. J. Dairy Res. 87, 115–121. https://doi.org/10.1017/S0022029920000564.
Placzek, M., Christoph-Schulz, I., Barth, K., 2021. Public attitude towards cow-calf separation and other common practices of calf rearing in dairy farming – a review. Org. Agr. 11, 41–50. https://doi.org/10.1007/s13165-020-00321-3.
Prenner, M.L., Prgomet, C., Sauerwein, H., Pfaffl, M.W., Broz, J., Schwarz, F.J., 2007. Effects of lactoferrin feeding on growth, feed intake and health of calves. Arch. Anim. Nutr. 61, 20–30. https://doi.org/10.1080/17450390600973675.
R Core Team, 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ⟨https://www.R-project.org/⟩.
Roth, B.A., Barth, K., Gygax, L., Hillmann, E., 2009. Influence of artificial vs. mother-bonded rearing on sucking behaviour, health and weight gain in calves. Appl. Anim. Behav. Sci. 119, 143–150. https://doi.org/10.1016/j.applanim.2009.03.004.
Selman, I.E., McEwan, A.D., Fisher, E.W., 1971. Studies on dairy calves allowed to suckle their dams at fixed times Post partum. Res. Vet. Sci. 12, 1–6. https://doi.org/10.1016/s0034-5288(18)34230-9.
Stott, G.H., Marx, D.B., Menefee, B.E., Nightengale, G.T., 1979. Colostral immunoglobulin transfer in calves. IV. Effect of suckling. J. Dairy Sci. 62, 1908–1913. https://doi.org/10.3168/jds.S0022-0302(79)83522-5.
Superti, F., 2020. Review. Lactoferrin from bovine milk: a protective companion for life. Nutrients 12, 2562. https://doi.org/10.3390/nu12092562.
Uetake, K., 2013. Newborn calf welfare: a review focusing on mortality rates. Anim. Sci. J. Anim. Sci. J. 84, 101–105. https://doi.org/10.1111/asj.12019.
Vaarst, M., Hellec, F., Verwer, C., Johanssen, J.R.E., Sørheim, K., 2020. Cow calf contact in dairy herds viewed from the perspectives of calves, cows, humans and the farming system. Farmers' perceptions and experiences related to dam-rearing systems. Landbauforsch. J. Sustain. Org. Agric. Syst. 70, 49–57. https://doi.org/10.3220/LBF1596195636000.
Veissier, I., Care, S., Pomies, D., 2013. Suckling, weaning, and the development of oral behaviours in dairy calves. Appl. Anim. Behav. Sci. 147, 11–18.
Waiblinger, S., Menke, C., Fölsch, D.W., 2003. Influences on the avoidance and approach behaviour of dairy cows towards humans on 35 farms. Appl. Anim. Behav. Sci. 84, 23–39. https://doi.org/10.1016/S0168-1591(03)00148-5.
Waiblinger, S., Wagner, K., Hillmann, E., Barth, K., 2020. Short- and long-term effects of rearing dairy calves with contact to their mother on their reactions towards humans. J. Dairy Res. 87, 148–153. https://doi.org/10.1017/S0022029920000576.
Weaver, D.M., Typer, J.W., VanMetre, D.C., Hostetler, D.E., Barrington, G.M., 2000. Passive transfer of colostral immunoglobulins in calves. J. Vet. Intern. Med. 14, 569–577. https://doi.org/10.1111/j.1939-1676.2000.tb02278.x.
Windschnurer, I., Schmied, C., Boivin, X., Waiblinger, S., 2008. Reliability and inter-test relationship of tests for on-farm assessment of dairy cows' relationship to humans. Appl. Anim. Behav. Sci. 114, 37–53. https://doi.org/10.1016/j.applanim.2008.01.017.