




Article

Good Handling Practices Have Positive Impacts on Dairy Calf Welfare

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Abstract: The objective was to evaluate the effects of good handling practices on dairy calf welfare. Forty-eight crossbred dairy calves were assigned to two treatments: conventional handling (CH): calves kept in individual pens, fed milk replacer in buckets without nipples and abruptly weaned; or good handling practices (GHP): calves kept in group pens, fed milk replacer in buckets with nipples, given daily tactile stimulation during feeding, and progressive weaning. Calf welfare was assessed from birth to 120 days of age, based on: health (plasma concentrations of glucose and IgG, and occurrences of diarrhea, pneumonia, tick-borne disease, or death); physiology (heart rate [HR], respiratory rate [RR], and rectal temperature [RT]); behavior (flight distance [FD], latencies for first movement [LM] and to hold the calf in a pen corner [LH], and total time a calf allowed touching [TTT]); and performance indicators (body weight, average daily gain, and weaning success at 70 days of age). Calves in the GHP treatment had a lower HR at 30 days of age, shorter FD and LH, longer TTT, and lower RR and RT than CH ($p < 0.05$). However, health, deaths and performance indicators did not differ ($p > 0.05$) between treatments. Based on various indicators, GHP improved dairy calf welfare.

Keywords: behavior; dairy cattle; health; human–animal relationship; performance



Citation: Silva-Antunes, L.C.M.; Ceballos, M.C.; Negrão, J.A.; Paranhos da Costa, M.J.R. Good Handling Practices Have Positive Impacts on Dairy Calf Welfare. *Dairy* **2024**, *5*, 295–307. <https://doi.org/10.3390/dairy5020024>

Academic Editor: Andrea Vitali

Received: 22 February 2024

Revised: 9 May 2024

Accepted: 10 May 2024

Published: 15 May 2024



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1. Introduction

The welfare of farm animals has been frequently studied [1], particularly when animals are directly dependent on basic care provided by humans, e.g., dairy calves [2]. Unfortunately, management failures are still common, compromising animal welfare [3–5] and causing high rates of morbidity and mortality [6], with serious ethical and economic implications [7–10]. In various European surveys, dairy calf mortality ranged from 2.0 to 8.0% [6,11–13]. In Brazil, these values were more variable, ranging from 2.33% [14] to 18.79% [15]. Thus, there is an urgent need to review dairy calf management methods and as an initial step, to evaluate animal welfare under various kinds of management [2].

Although it is not possible to eliminate calf death, there must be a commitment to minimize its occurrence [16]. Therefore, identification of causes of calf death and strategies to eliminate or, at least, minimize them, is needed. Infectious diseases, in particular diarrhea [17] and pneumonia [18], are the main causes of death in dairy calves. These diseases may be associated with failures of transfer of passive immunity, usually due to deficiencies in colostrum feeding. Thus, the first step to reducing illness and death is to

ensure calves ingest an adequate amount of good-quality colostrum in their first hours of life [19,20].

There are also environmental causes that predispose calves to infectious and contagious diseases. Consequently, there are recommendations for managing dairy calves in individual pens [11] or in individual outdoor shelters [21] to reduce the spread of infectious diseases. However, dairy calves kept in social isolation have more welfare problems compared to those managed in groups [22,23]. Managing dairy calves in groups with an increased frequency of interactions with handlers had positive results [15,24,25], demonstrating that calves managed under such conditions were less fearful toward humans or when placed in an unfamiliar environment.

Nevertheless, other management elements deserve attention when seeking to improve dairy calf welfare, including those related to milk-feeding and weaning [26,27]. In Brazil, milk is usually fed in buckets without nipples, generating behavioral problems that can be avoided by feeding milk in buckets with nipples [28,29]. It is expected that there will be variations among calves in their dependence on milk intake, as some adapt more quickly than others to solid feed [30]. However, in Brazil, this individual variation is generally not considered when weaning dairy calves, which is generally carried out abruptly at 6 to 8 weeks of age [31]. One possibility to reduce stress caused by early and abrupt weaning is to perform it gradually [32], thereby promoting increased solid feed intake [27].

It is evident that good handling practices have the potential to enhance dairy calf welfare. To our knowledge, no previous study assessed the effects of the combined adoption of several practices on dairy calf welfare using health, physiological, behavioral, and performance indicators. Our objective was to compare the impacts on dairy calf welfare of implementing a set of good handling practices (calves kept in groups, feeding milk in buckets with nipples, daily positive tactile stimulation, and gradual weaning) compared to traditional rearing practices used in Brazil. Our hypothesis is that the combined action of good handling practices improves dairy calf welfare.

2. Materials and Methods

This study was reviewed and approved by the Committee for Ethical Use of Animals, of the Faculty of Agricultural and Veterinary Sciences, São Paulo State University, Jaboticabal, SP, Brazil (Protocol 010936/11).

The research was conducted on a private farm in the municipality of Jaboticabal (21°15'17" S and 48°19'20" W, with an average altitude of 607 metres above sea level), São Paulo state, Brazil. Average minimum and maximum air temperatures were 12.5 and 33.5 °C. Forty-eight crossbred male dairy calves (Gir × Holstein and Jersey × Holstein) were sourced from three commercial farms located within 41 km of the farm where the experiment was conducted.

2.1. Experimental Protocol

Calves were >30 kg at birth (eutocic calving), from dams with good quality colostrum, based on its density in a bovine colostrum densimeter (Nasco Farm and Ranch bovine colostrometer, Fort Atkinson, WI, USA). Calves were separated from their dams up to 2 h after birth and received 2 L of colostrum (by suckling bottles) within 3 h after birth, plus an additional 2 L up to 12 h after birth. Newborn care, including navel disinfection, calf identification (numbered collar) and assistance with the first feeding, were all performed on the farm where calves were born; the calves remained there for the first 3 days of life, receiving colostrum *ad libitum*.

After being separated from their dams, calves were housed in individual pens and on the fourth day of life were transported to the farm where the experiment was conducted. They were allocated alternately, according to birth order and farm of origin, into two treatments (24 calves per treatment): 1. Conventional handling (CH), calves housed in individual mobile pens ("casinha tropical" [11]), where they were restrained (with a leather collar) and a 2 m leash. They were fed milk according to the farm protocol, receiving

4 L of milk replacer per day (Sprayfo Violeta[®], Trouw Nutrition Brasil, Campinas, SP, Brazil) in buckets without nipples, divided into two meals. Weaning was performed when calves reached 70 days of age and body weight (BW) \geq 70 kg; when calves did not meet the weaning weight criteria, they continued receiving 1 L of milk replacer per day until they reached the stipulated BW; or 2. Good handling practices (GHP), calves housed in collective pens ($\geq 3 \text{ m}^2/\text{calf}$) and with access to a paddock between morning and afternoon milk-feedings, from the fourth day of life, calves received 5 L of milk replacer (Sprayfo Violeta[®], Trouw Nutrition Brasil, Campinas, SP, Brazil) per day in buckets with nipples, which was gradually reduced to 4 L when calves reached 35 days of age, 3 L from 56 days of age, and 2 L per day from 61 days of age. During this interval, calves were fed milk twice a day, divided into two meals, with equal portions. From the 66th day of age, calves received only 1 L of milk replacer, in a single milk-feeding, until weaning. In addition, GHP calves received tactile stimulation (brushing) for 2.5 min during milk feeding, totalling 5 min/day. Brushing was performed with a soft brush, providing tactile stimulation of the back, ribs and upper hind legs. All handling procedures were performed by the same person. The weaning criterion was the same used for the CH group.

Calves from both groups were fed milk twice daily, at 07:00 and 15:00 h. Water and hay were offered ad libitum, with commercial concentrate (Guabitech Rumileite[®], Guabi Nutrição e Saúde Animal, Sales de Oliveira, SP, Brazil) according to consumption, with a maximum of 1.0 kg/calf/day. Milk quantity per calf was 268 and 270 litres for CH and GHP, respectively, except for those that did not reach the established weaning criteria and were fed milk for a longer interval. Calves in the GHP group received an additional 1 L of milk replacer during the first 35 days of age.

The total amount of milk replacer each calf was fed, and weaning criteria were based on the most common strategy adopted on Brazilian dairy farms. After weaning, all calves were kept in paddocks with Tifton 85 grass (*Cynodon* spp.) and fed 1.0 kg of commercial concentrate (Guabitech Rumileite[®], Guabi Nutrição e Saúde Animal, Sales de Oliveira, SP, Brazil) per day in two meals. Mineral supplements and water were available ad libitum.

2.2. Animal Welfare Assessment

Sixteen indicators were used to assess welfare (Table 1), being recorded when calves completed 30, 60, 90 and 120 days of age, except for plasma glucose and IgG concentrations, with blood samples also collected at 24 h of age. Body weight was also recorded at weaning.

Table 1. Dairy calf welfare indicators defined as dependent variables (health, physiology, behavior, and performance indicators).

Animal Welfare Indicators (Dependent Variables)
Health indicators
Plasma glucose concentration (Glucose, mg/dL)
Plasma IgG concentration (IgG, mg/mL)
Occurrence of diarrhea (Diarrhea, yes or no)
Occurrence of pneumonia (Pneumonia, yes or no)
Occurrence of tick-borne disease (TBD, yes or no)
Occurrence of death (Death, alive or dead)
Physiology indicators
Heart rate (HR, bpm)
Respiratory rate (RR, breaths/min)
Rectal temperature (RT, °C)
Behavior indicators
Flight distance (FD, m)
Latency for the calf's first movement during a docility test (LM, s)
Latency to hold the calf in a pen corner during a docility test (LH, s)
Total time that the calf allowed to be touched during a docility test (TTT, s)
Performance indicators
Body weight (BW, kg) at 30, 60, weaning, 90, and 120 days of age
Average daily gain (ADG, kg/day), from birth to 30 days and from 30–60, 60–90, and 90–120 days of age
Weaning success at 70 days of age (WS, yes or no)

2.2.1. Health Indicators

Blood samples (5 mL) from 8 calves per treatment (randomly selected) were collected (jugular venipuncture) five times, within the first 24 h of life and at 30, 60, 90, and 120 days of age. Samples were always collected just after the morning milk feeding. Two blood collection tubes (BD, Vacutainer™) with anticoagulant (EDTA) were used on each sample day to measure plasma concentrations of IgG and glucose. Immediately after blood collection, samples were centrifuged to obtain plasma and frozen (−20 °C) until analyzed.

Total IgG classes were evaluated based on enzyme-linked immunosorbent assays (ELISA) using commercial kits (Bethyl Laboratories, Montgomery, TX, USA), as described [33]. An enzymatic glucose oxidase/peroxidase method (Glucose GOD-PAP, Liquid Stable) [34] was used to assess plasma glucose concentrations. Detection limits and inter- and intra-assay coefficients were 2.02 ng/mL, 6% and 9%, respectively for IgG, and 6.2 mg/dL, 2.6 and 4% for glucose.

Calf health was monitored throughout the study, including clinical signs of diarrhea, pneumonia and tick-borne disease (bovine babesiosis and anaplasmosis complex). No cases of pneumonia or tick-borne disease were recorded. Diarrhea (recorded when pasty or liquid feces were present or if the perineum was soiled) and deaths were recorded using a binomial scale, assigning a score of “0” to calves that did not have diarrhea and “1” to those that did it, and “0” to surviving calves and “1” to those that died.

2.2.2. Physiological Indicators

Three physiological measurements were performed once monthly, after the morning feeding, for calves without clinical signs of disease. However, calves with clinical signs of diarrhea on assessment days were not examined, and the examination was delayed until the calf recovered, on average, after 3 to 5 days. Physiological indicators were recorded in the following order: respiratory rate (RR, breaths/min), assessed by counting the number of respiratory cycles per minute based on flank movements (observing the animal from behind); heart rate (HR, bpm), obtained by cardiac auscultation; and rectal temperature (RT, °C), measured using a digital veterinary thermometer (Incoterm®, Porto Alegre, RS, Brazil).

2.2.3. Behavioral Indicators

Four behavioral indicators were measured: flight distance (FD, m); latency for the first movement during docility test (LM, s); latency to hold the calf in one corner of the pen during docility test (LH, s); and the total time that the calf allowed touching during the docility test, to a maximum interval of 1 min (TTT, s).

FD measures the minimum distance an animal allows an observer to approach before it moves away [35] and was measured in a pen unfamiliar to the calves. On each assessment day, three measurements of FD were performed for each calf, and the average was used for analyses.

The docility test [36] was conducted in two pens, where calves could not see other calves, by combining a series of actions generating three variables (LM, LH and TTT). Calves were calmly moved in groups to an unfamiliar pen and then driven, one by one, by the same person (familiar) to another pen (test pen), where the following procedures were performed: the calf remained in the test pen for 30 s (for familiarisation), then an unfamiliar person entered the pen, remaining stationary in the pen centre for 30 s. LM measured the latency of a calf's first movement within this interval. After 30 s, the person tried to keep the calf in one corner of the pen, recording the time required to perform it (LH). In situations where the person was able to hold the calf in one of the corners of the pen for 30 s, the person approached even more and tried to touch the calf; if they succeeded, the total time the calf allowed touching was recorded (TTT, considering a maximum time of 1 min). The 30-s interval for initiating docility tests was determined based on a preliminary assessment, recording the interval for calves to cease exploratory behaviour in the pen where docility tests were conducted.

2.2.4. Performance Indicators

Calves were weighed shortly after birth and every 30 days until 120 days of age, defining variable body weight (BW, kg). Average daily weight gain (ADG, in kg/day) was calculated every 30 days, always considering initial body weight. In addition, all calves were weighed at weaning, recording those that succeeded or failed to meet established weaning criteria at 70 days (70 kg BW and minimum daily intake of 700 g concentrate/day), defining weaning success (WS, yes or no).

2.3. Statistical Analyses

Statistical analyses were conducted using R software with an integrated development environment RStudio (R version 4.3.0, 2023). The normality of residuals was tested using a Shapiro–Wilk test. To select the most appropriate structure of (co)variance of residuals, Akaike information criteria (AIC) and Schwarz Bayesian (BIC) were used [37]. Statistical analyses of physiology, behavior, and performance (except WS) indicators included linear mixed models for repeated measures using a MIXED procedure (GLMM). The model included treatment (GHP and CH), calf age (30, 60, 90, and 120 days of age) and the interaction between both as fixed effects. Calf (subject) was included as a random effect to control for repeated measures. Birth weight was included as a covariate for performance indicators analyses (except WS). Tukey's test was used to compare adjusted means, with $p < 0.05$ considered significant.

Health indicators and WS were analyzed using the GENMOD procedure, assuming binomial data distribution with a probit link function to an adjacent normal distribution. The model included treatment as a fixed effect. Odds ratios were calculated to estimate weaning success (WS) relative chance and relative risk of diarrhea, and deaths between treatments (GHP and CH). Reference values included the lowest occurrence of WS, but the highest occurrences of diarrhea and death.

3. Results

A summary of all statistical analyses is included in Supplementary Table S1.

3.1. Health Indicators

There was no effect of the interaction between treatment and calf age ($p > 0.05$) nor of treatments ($p > 0.05$) on plasma glucose concentrations. However, plasma glucose concentrations differed among calf ages ($p < 0.001$), lowest on day 1 (52.70 ± 6.31 mg/dL), compared to other ages (108.60 ± 6.53 , 108.60 ± 6.31 , 93.6 ± 6.31 and 101.80 ± 6.53 mg/dL, for 30, 60, 90 and 120 days of age, respectively), with no significant difference among the latter four ages.

There was an interaction between treatment and calf age ($p = 0.03$, Figure 1) for IgG.

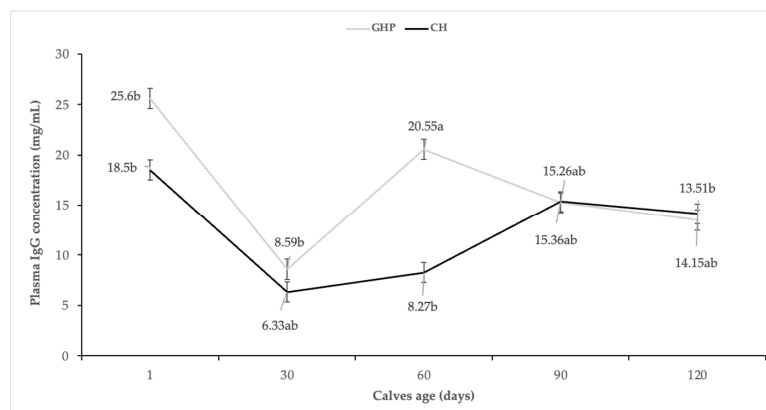


Figure 1. Adjusted means and SEM of plasma IgG in dairy calves according to treatment (GHP = Good handling practices; CH = Conventional handling) and age (1, 30, 60, 90, and 120 days). Means without a common letter differed between treatments and calf age ($p < 0.05$).

There was no effect of treatment on the occurrence of diarrhea ($p = 0.832$) or death ($p = 0.357$), although the mortality rate was much higher in CH than in GHP (12.00 and 4.35%, respectively, Table 2).

Table 2. Relative frequencies, odds ratios (OR) and 95% confidence intervals (CI) for occurrences of diarrhea and death in dairy calves between 8 and 120 d of age.

Health Indicators	Frequency (%)	OR (SEM)	CI (95%)	<i>p</i> Values
Diarrhea				
Intercept	-	2.00	0.06–0.56	0.005
GHP	15.00	0.84	0.15–4.33	0.832
CH	17.40	Ref.	Ref.	Ref.
Death				
Intercept	-	0.14	0.03–0.39	0.001
GHP	4.35	0.33	0.02–2.83	0.357
CH	12.00	Ref.	Ref.	Ref.

GHP = Good handling practices, CH = Conventional handling and Ref. = reference class.

3.2. Physiological Indicators

There were no interactions ($p > 0.05$) between treatment and calf age on RR or RT. Calf age had an effect on RT ($F = 4.82$, $p = 0.003$), with the highest mean at 30 d (39.29 ± 0.07 °C) compared to all other ages (38.96, 38.99, 39.01 °C for 60, 90 and 120 days of age, respectively; SEM = 0.07 for all). Treatment affected (Mean \pm SEM for GHP and CH, respectively) RR, breaths/min (45.50 ± 2.19 vs. 51.70 ± 2.25 , $F = 4.09$, $p = 0.05$) and RT, °C (38.98 ± 0.05 vs. 39.15 ± 0.05 , $F = 5.18$, $p = 0.03$). There was an interaction between treatment and calf age for HR ($F = 2.78$; $p = 0.04$, Table 3).

Table 3. Adjusted means \pm SEM for HR in calves, according to the interaction between treatment and age.

Treatment	Calf Age (Days)			
	30	60	90	120
HR, bpm				
GHP	$80.00b \pm 3.67$	$85.70b \pm 3.67$	$84.50b \pm 3.67$	$82.20b \pm 3.76$
CH	$104.60a \pm 3.77$	$96.20ab \pm 3.77$	$93.00ab \pm 3.87$	$95.30ab \pm 3.87$

GHP = Good handling practices, CH = Conventional handling, HR = heart rate (bpm: beats per minute). Means without a common letter differed between treatments and calf age ($p < 0.05$).

3.3. Behavioral Indicators

There was no interaction ($p > 0.05$) between treatment and calf age for any behavior variable. However, treatment affected FD ($F = 18.54$, $p < 0.001$), LH ($F = 8.85$, $p = 0.005$) and TTT ($F = 9.36$, $p = 0.003$), but not LM ($F = 0.61$; $p = 0.44$). Calves from GHP had lower FD and LH, and higher TTT ($p < 0.05$) compared to CH calves (Table 4). Calf age affected LM ($F = 4.60$, $p = 0.004$), LH ($F = 9.92$, $p < 0.001$) and TTT ($F = 3.79$, $p = 0.01$) (Table 4).

Table 4. Adjusted means and SEM for behavior indicators measured in dairy calves according to treatment and calf age.

Independent Variable	Behavioral Indicators			
	FD	LM	LH	TTT
Treatment				
GHP	$0.45b \pm 0.09$	$8.59a \pm 1.59$	$45.10b \pm 5.19$	$53.60a \pm 2.78$
CH	$1.02a \pm 0.09$	$10.30a \pm 1.59$	$66.40a \pm 5.28$	$41.30b \pm 3.10$

Table 4. Cont.

Independent Variable	Behavioral Indicators			
	FD	LM	LH	TTT
Calf age				
30	0.62b \pm 0.09	5.28b \pm 1.62	37.10b \pm 6.60	52.20a \pm 2.99
60	0.90a \pm 0.09	9.27a \pm 1.58	38.90b \pm 6.87	52.60a \pm 3.41
90	0.76ab \pm 0.09	11.52a \pm 1.69	73.40a \pm 6.98	41.90b \pm 3.50
120	0.72ab \pm 0.09	11.72a \pm 1.83	41.90b \pm 7.20	43.00b \pm 3.63

GHP = Good handling practices, CH = Conventional handling, FD = flight distance, LM = latency for the calf's first movement during the docility test, LH = latency to hold the calf in one corner of the pen during the docility test and TTT = total time that the calf allowed to be touched during the docility test. Means without a common letter in the same independent variable and column differed between treatment and calf age ($p < 0.05$).

3.4. Performance Indicators

Birth weight did not differ between treatments ($F = 0.08$; $p = 0.77$), with mean \pm SEM for GHP 39.3 ± 0.58 and CH 39.6 ± 0.59 kg. There was no effect of the interaction between treatment and calf age, nor of treatment ($p > 0.05$) on BW and ADG. As expected, there was an effect of calf age on BW and ADG (Table 5). There was no effect of treatment on WS ($p > 0.05$).

Table 5. Adjusted means \pm SEM for performance indicators according to calf age.

Performance Indicators	Calf Age (Days)			
	30	60	90	120
BW	46.50d \pm 1.26	59.70c \pm 1.26	85.90b \pm 1.26	105.20a \pm 1.26
ADG	0.230c \pm 0.02	0.340b \pm 0.02	0.520a \pm 0.02	0.550a \pm 0.02

BW = body weight (kg) and ADG = average daily gain (kg/day). Means without a common letter in the same line differed among calf ages ($p < 0.05$).

4. Discussion

We evaluated a combination of good handling practices (GHP: calves kept in groups, fed milk replacer in buckets with nipples, given daily brushing and gradual weaning) aiming to improve their welfare. Most of our results supported the hypothesis that GHP improves dairy calf welfare compared to conventional handling (CH). The GHP calves had shorter FD and LH, longer TTT and lower RR, RT and HR at 30 d of age. The concept of assessing the combined effects of GHP was based on potential synergies [38] yielding enhanced outcomes for calf welfare, surpassing the benefits of adopting each practice separately. The impetus for this study was a report by Levine (2001) [39] that rat pups deprived of maternal care between 4 and 14 days of age had higher basal corticosterone concentrations and reacted robustly to mild stress, with higher corticosterone and ACTH, compared to pups in contact with their dams. In that study, maternal care (tactile stimulation, feeding and passive contact) was reported as critical for the pup's hypothalamic–pituitary–adrenal (HPA) axis regulation [39], and tactile stimulation inhibited most brain changes occurring after maternal deprivation. Thus, we included tactile stimulation as a good handling practice for dairy calves separated from their dams shortly after birth. Furthermore, the motivation for calves to suckle [40,41] justified buckets with nipples, increasing milk replacer consumption, albeit at a slower rate [40], and reducing abnormal sucking behaviours [41].

Cattle are herd animals and in natural conditions with their dams in a herd, calves experience a complex social environment, which is altered when they are isolated. According to Costa et al. [42], raising calves in isolation results in deficient social skills, difficulties in dealing with new situations and specific cognitive deficits. However, the authors [42] highlighted that the effects of group housing on calves' health are contradictory, with some studies reporting an increased risk of disease and others no difference or even better health outcomes for grouped calves. Based on their review [42], group housing improved solid feed consumption in dairy calves, whereas weight gain and health risks associated with

group housing can be mitigated with appropriate management. Our results corroborated this statement since there were no significant differences between GHP and CH calves in the occurrences of diarrhea, pneumonia, tick-borne disease or death, although the latter was numerically more frequent in CH calves.

Weaning is an important cause of stress in dairy calves [43,44] and if performed poorly, can harm both calf performance and welfare [43]. However, this can be mitigated with gradual weaning. Inconsistent results about this practice have been reported, with no effect on calf performance [44] or with improved growth and feed efficiency of dairy calves [45].

Collectively, these reports formed the basis for us to evaluate the combined effects of several good handling practices on calf welfare, rather than assessing them individually.

4.1. Health Indicators

Regarding immunity status, there was an interaction between treatment and calf age for IgG concentration. Plasma IgG concentrations in the first 24 h of life in calves from both treatments (25.6 and 18.5 mg/mL for GHP and CH, respectively) exceeded the minimum recommended for successful transfer in this period (IgG > 10 mg/mL) [46]. Therefore, the transfer of passive immunity was successful, regardless of treatment, which was expected, due to high-quality colostrum and adequate intake in the first 3 h of life.

As expected, when neonate calves first handling procedures are performed properly, higher values of IgG were achieved on the first day of life and subsequently reduced in both treatments [47]. Blood IgG concentrations peak within 24 h after birth and then decline to ~30 days of age [48], as observed in the present study. Variations in IgG concentrations over time for calves in this study were consistent with those obtained by Feitosa et al. [49] and Leal et al. [50] with Holstein calves. However, at 60 days of age, GHP calves had higher ($p = 0.03$) IgG concentrations, more than double those of CH calves. After 30 d age, synthesis of endogenous immunoglobulin begins to increase IgG concentration, reflecting active responsiveness of the immune system [48]. Nevertheless, after 90 d, both treatments had the same IgG concentrations. Calves are considered to reach full immune system activity by 6 months of age, when they can respond similarly to adults [48,51].

Adoption of GHP did not increase the risk of diarrhea occurrence compared to CH. This disagrees with the expectation that group housing would increase the risk of infectious diseases, such as diarrhea, due to proximity between animals [21,52]. We inferred that the daily tactile stimulation improved human–calf relationship quality and increased the time dedicated by the handler for calf care, important factors affecting calf welfare, and arguably more important than the type of facilities. Closer interactions with calves can facilitate recognition of apathy and weakness and changes in feeding behavior, which could result from diseases such as diarrhea [15]. Daily physical contact with calves, as performed in GHP calves, may reduce disease development, since initial clinical signs are more likely to be noticed. In addition to the closer human–animal interactions with GHP calves, gradual weaning may also have contributed to this result, corroborating the outcomes of Khan et al. [32] and Sweeney et al. [27], who reported lower levels of stress in calves that had gradual weaning, which may be associated with good body condition due to higher concentrate intake, with direct benefits for calf health.

Although the occurrence of calf deaths did not statistically differ between treatments, most deaths were associated with diarrhea, and the frequency of death was nearly three times higher in CH versus GHP calves, with productivity implications. Plasma glucose concentrations did not differ between treatments, with indications that calves from both treatments were well-fed throughout the study period, with values being in the normal range (80 to 120 mg/dL) [53].

4.2. Physiological Indicators

The GHP calves had lower RR and RT mean than CH calves; the latter had HR and RR means slightly above the normal ranges for young cattle (60 to 90 beats per minute and 20

to 50 breaths/min [54]). However, restraint to assess physiological indicators is probably more challenging for CH calves, as they are less accustomed to human–animal interactions.

There are clear associations among human–animal interactions, stress, and physiological responses [55]. A possible explanation for the differences in HR and RT between groups could be that GHP calves associated their interaction with humans with positive and rewarding experiences, e.g., brushing and suckling nipples, which may have improved human–animal relationships in GHP calves. In sheep, positive handler–animal interactions decrease stress, with lower blood cortisol concentrations and HR [56]. This physiological response is supported by their behavior, as GHP calves had lower fear reactions [57] compared to CH calves. Fear of humans negatively affects animal welfare [55,58]. Adoption of GHP may have contributed to the calves being calmer in the presence of humans compared to those receiving CH procedures.

4.3. Behavioral Indicators

Calves in the GHP group had lower FD and LH and higher TTT means. Positive and rewarding experiences resulting from human interaction directly influence behavior such as those evaluated here [59]. The FD is considered one of the most reliable methods to assess cattle fear of humans [60]. Similar results were reported for dairy calves when positive practices, such as talking to them or touching them, and group housing, improved behavioral responses [24].

Less fearful behavioral responses in GHP calves may have been related to the closer and positive contact between humans and calves, including brushing during milk feeding [15], resulting in lower FD and LH and higher TTT. Gently touching and stroking has the potential to improve dairy calves' welfare, by reducing fear responses toward humans [61]. In addition to the human–animal relationship, positive behavioral reactions have also been described in lambs managed in group pens [62]. Our results corroborated the interpretations of Lensink et al. [63], who concluded that the notion that calves managed in groups are more difficult to handle and more aggressive than calves managed alone is mistaken. Thus, we concluded that GHP reduced calves' fear of humans, facilitating daily handling routines.

4.4. Performance Indicators

BW and ADG did not differ between treatments at any age. However, the literature is contradictory. In some studies, calves kept in individual pens had better ADG [64], whereas in others, calves reared in groups had higher ADG [65–69]. Most studies mentioned above indicated that group housing improved dairy calf welfare and promoted initial searches for solid feed. In contrast, calves in individual pens reduced disease transmission, which was not observed herein.

Finally, from an animal welfare perspective, it is recommended to use several indicators to have a reliable assessment of the animal welfare state at a given moment [70,71]. In the present study, we used several indicators and demonstrated that applying GHP to dairy calf management generally promoted a higher level of welfare compared to calves managed conventionally. However, because the GHP group was a set of practices that aimed to improve calf welfare, it is uncertain which of these practices had the greatest influence.

5. Conclusions

This study demonstrated positive effects on dairy calf welfare by adopting a series of handling procedures, defined as GHP, including keeping calves in groups, feeding milk replacer from buckets with nipples, daily brushing, and gradual weaning. Based on physiology and behavior, GHP improved welfare compared to calves managed under conventional handling conditions, without impairing health and productivity, and highlighted the importance of reconsidering a paradigm shift, i.e., it is not necessary to isolate dairy calves to ensure good health.

Results of this study are relevant for the dairy cattle industry and indicate the need for studies that advance knowledge about the individual role of each GHP procedure, to improve dairy calf welfare and dairy farm productivity.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/dairy5020024/s1>, Table S1. Summary of statistical analyses outcomes, including all dependent variables (DF = degrees of freedom).

Author Contributions: Conceptualization, L.C.M.S.-A. and M.J.R.P.d.C.; Methodology, L.C.M.S.-A., J.A.N. and M.J.R.P.d.C.; Formal Analysis, L.C.M.S.-A. and M.J.R.P.d.C.; Investigation, L.C.M.S.-A. and M.J.R.P.d.C.; Resources, L.C.M.S.-A. and M.J.R.P.d.C.; Data Curation, L.C.M.S.-A.; Writing—Original Draft Preparation, L.C.M.S.-A., M.C.C., and J.A.N.; Writing—Review and Editing, M.C.C., M.J.R.P.d.C., and J.A.N.; Supervision, M.J.R.P.d.C. and J.A.N.; Project Administration, L.C.M.S.-A.; Funding Acquisition, M.J.R.P.d.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by The São Paulo Research Foundation (FAPESP), projects 2011/00388-3 and 2011/00368-2.

Institutional Review Board Statement: This study was reviewed and approved by the Committee for Ethical Use of Animals of the Faculty of Agricultural and Veterinary Sciences, São Paulo State University, Jaboticabal, SP, Brazil (Protocol 010936/11).

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Acknowledgments: We are grateful to Mauricio Vital, José Nelson and Toni Ângelo, for their strong support to carry out this study; to FAI do Brasil (especially Murilo Henrique Quintiliano, Cleverson and Nadir Bornatte) for allowing us to carry out this research in its facilities and help during the data collection. We also thank Luciana Pontes da Silva for her help during data collection, Joseph Kaled Grajales Cedeño, for support in statistical analyses, and to John P. Kastelic (University of Calgary) for correcting style and English grammar.

Conflicts of Interest: The authors declare no conflict of interest.

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