THESIS

STRATEGIES TO ENHANCE HEALTH AND WELL-BEING OF DAIRY CALVES: EXPLORING THE USE OF PREBIOTICS AND ENVIRONMENTAL ENRICHMENT

Submitted by

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2018

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ABSTRACT

STRATEGIES TO ENHANCE HEALTH AND WELL-BEING OF DAIRY CALVES: EXPLORING THE USE OF PREBIOTICS AND ENVIRONMENTAL ENRICHMENT.

Replacement dairy calves and heifers represent the future for dairy producers. However, rearing healthy animals is not an easy task, since the first day of life calves encounter stressors and management systems that might impact their future health and survival. It is a fundamental production management need to identify and understand these critical windows of stress in the pre-weaned and weaned life in order to create strategies that benefit young animals. This thesis is focused to exploring alternatives for prevention and treatment of neonatal diarrhea and measuring changes in behavior and health when an enriched environment is provided in the first weeks after weaning and grouping in collective pens.

In chapter 1, a brief literature review related with health and well-being of dairy calves is presented.

The objective of the experiment presented in chapter 2 is to evaluate the addition of stabilized rice bran (SRB) in the milk of neonatal Holstein calves for a period of 28 days and its effects on health, immunity and performance. A paired comparison design was performed with a control (n=45) and a treatment group (n=45). The variables analyzed were neonatal diarrhea presentation, time to recovery from a moderate diarrhea episode, animal removal, concentration of IgA in feces, and average daily gain (ADG). After the treatment period was completed, health, disease presentation, and animal removal was analyzed until weaning through the use of farm

records. No differences were found for any of the variables studied in the 28 days of SRB addition or in the follow up until weaning.

Chapter 3 is focused on the time of weaning, when dairy calves are grouped for the first time in their lives. The objective was to evaluate the effects of an automated grooming brush on health, behavior and performance through a paired comparison design, with one treatment group (n= 81) and one control group (n=81). Four groups of calves were housed in pens of 19 to 22 animals for 3 weeks. One automated brush was placed per treatment pen. Individual behavior data was obtained through the use of 3-D accelerometer sensors. The variables analyzed included disease presentation, time to first clinical disease, animal removal due to disease, and ADG. The 3-D sensor data allowed analysis of "eating", "rumination", "not active", "active", and "high active" behavioral activities, as daily averages and hourly averages. No differences were found for animal removal, time to first clinical disease, or ADG. A tendency was found for the presentation of diseases, indicating that control calves were more likely to be detected sick. Additionally, significant differences were found for "eating" time in favor of treatment calves (P=0.01), and "not active" time in favor of control calves (P=0.014) by day. Hourly differences were found for the variables "eating" time; "not active" time , and "high active" Indicating that treatment calves spent less time "not active" and more time "eating" than control calves by day and more time high active at certain hours than control calves.

From our results, the addition of SRB in milk of pre-weaned calves had no an effect on health, immunity or performance of pre-weaned calves. The presence of an automated brush in calves housed in collective pens had a positive effect in the behavior of weaned calves.

ACKNOWLEDGEMENTS

I would like to thank to my advisor Dr. Pablo Pinedo and all the members of Dairy Management System Group for their friendship, guidance and support throughout these years. Additionally to USDA-NIFA OREI for the award number 2016-51300-25734.

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CHAPTER 1: LITERATURE REVIEW

Dairy calf rearing in the United States

Different calf rearing systems have been described in the U.S. dairy farms (USDA, 2016). Each system is selected depending on farm size, resources, environment and location of the farm. For example, there are farms that rear calves in individual hutches, in pairs or in groups. Some farms feed milk in bottles and others in buckets and some farms provide milk replacers while others feed saleable milk or pasteurized not saleable milk, etc. However, the objective is always to optimize calf health and the success of the rearing period in order to maintain profitability

In general terms, calves are separated from the dam almost immediately after birth and fed with fresh, frozen or powder colostrum. Overall, 52.7% of farms hand fed colostrum to calves and 15.5% of these operations evaluate colostrum quality with colostometer or brix refractometer before delivery to calves (USDA, 2016). Also, the navel is disinfected at this stage. Usually, newborn calves are kept in a specific area designed for them for around 24 hours after birth to allow for constant observation and colostrum feeding until completing the amounts specified for each farm.

The most common housing system in the U.S. for pre-weaned calves is the outside individual hutch (37.9% of all operations) followed by indoors housing in an unheated barn (25.1% of all operations), depending on the region and weather conditions (USDA, 2016; Pempek et al., 2016). For weaned calves, 1.4% of operations housed them individually. Most operations provide grouped housing and depending on the region it might be pen/dry lot with barn/shed or multiple animals inside area/barn (USDA, 2016).

Water is on average offered starting at 17 days of age; large operations provide water earlier than medium and small farms. In addition, calf starter is provided at an average of 11 days of life, but large operations offer starter when calves are 6.3 days old (USDA, 2016). Although, it has been recommended that water should be offered since day 1 and calf starter since 4 days of life (BAMN, 2017). Dehorning and vaccination are common practices during the pre-weaned period, with the most common dehorning method being hot iron, performed at 7.1 weeks of age (USDA, 2018). Most farms have protocols detailing the age of vaccinations and dehorning. In 2011, farm protocols were created by the management in 31.1% and by veterinarians in 20% of all size operations (USDA, 2012). On average, weaning is completed at 9 weeks of life (USDA, 2016). The major criterion for weaning is age, followed by amount of calf starter consumption, although, it has been recommended by Bovine Alliance on Management and Nutrition (BAMN, 2017) that the main criteria should be healthy animals with a consumption of 1.3 Kg of starter for 3 consecutive days. To reduce stress, a step down weaning is preferred and calves should stay in the same housing for a week after weaning completion before transference into group housing. After weaning, calves are housed in groups and fiber is added to the diet in small amounts, in conjunction with calf starter (BAMN, 2017). Vaccinations are provided at this stage as a common practice.

Health challenges

Calf management and health are a constant challenge for dairy producers and calf raisers. An efficient system from gestation to breeding allows heifers to target the expected objectives set by the industry, such as low mortality and incidence of diseases, as well as adequate average daily gain (ADG), weaning time, age at first service and calving. However, enhancing the health of young calves and preventing diseases is not an easy task, as stressors and exposure to pathogens are intrinsic to dairy calves rearing systems (Hubert and Moisá, 2015).

Stress might negatively impact immunity and health and sick animals represent a challenge for producers, not only for the extra time spent treating them or the associated costs, but also for negative impact on the future performance of animals (Place at al., 1998; Heinrichs and Heinrichs, 2011; Soberon and Van Amburgh, 2013; Hubert and Moisá, 2015)

Therefore, it is fundamental to identify critical periods in order to improve health, immunity, and performance (Hulbert and Moisá, 2015). The critical windows described by Hulbert and Moisá (2015) are the first day of life, including birth and colostrum management; the first or second week after birth, when calves are highly susceptible to digestive diseases; and the weaning and commingling period. In addition, transportation to the rearing area or off site operation during the first days of life and painful procedures like disbudding and castration are defined as potential stressors in the pre-weaned period that might affect health and performance.

Alternatives to antimicrobials and enhancement of the immune system

Diarrhea is the main cause of disease, treatments, and deaths in pre-weaned dairy calves (Timmerman et al., 2005; McGuirk, 2008; Santos et al., 2015). In 2013 it was reported that 56.4% and 24% of deaths in pre-weaned calves were related to digestive and respiratory problems, respectively (USDA, 2018). Overall, 75.9% and 94.8% of calves suffering digestive or respiratory diseases were treated with antimicrobials. Conversely, the main cause of death in weaned heifers are respiratory problems (McGuirk, 2008), and 91.8% of sick heifers were treated with antimicrobials for this cause (USDA, 2018).

Considering this data, the restrictions in use of antimicrobials, and the recommendations from the Antimicrobial Resistance Action Plan present an urgent need to improve farm practices, developing new approaches to enhance immunity, reduce stress and prevent, and treat diseases in dairy calves.

One of the strategies to improve resistance and recovery from diarrhea and, therefore, to improve calf health is nutrition management. Pre and probiotics have been described as a preventive strategy and as a treatment for neonatal calf diarrhea (Heinrichs et al., 2009; Gosh and Mehla, 2012; Santos et al., 2015; Yutaka et al., 2015; Froehlich et al., 2017), although findings in published data are not consistent (Heinrichs et al., 2009; Ballou, 2011; Gosh and Mehla, 2012; Heinrichs et al., 2013; Kara et al., 2015; Froehlich et al., 2017; Wolfswinkel, 2017). This group of feed additives help to maintain a healthy gastrointestinal intestinal (GI) microbiota, which might lead to an improvement of the immune system, feed efficiency, and performance of dairy calves (Ballou, 2011; Yutaka et al., 2015; Wolfswinkel, 2017).

Probiotics have been defined as "live organisms that confer health benefits to the host when administered in adequate amounts"(FAO/WHO, 2001). Probiotics enhance intestinal health through the growth stimulation of the beneficial microbiota and by preventing the colonization of pathogens by competition. An additional action is the strengthening of mucosal surfaces and modulating of immune responses due to the production of short chain fatty acids as a consequence of fermentation (Henderson et al., 2012). Yukata et al. (2015) described the importance of introducing microorganisms that will not disturb the host microbes that are adapted to the GI environment. Yeast, fungi, and bacteria have been used as probiotics in ruminants.

On the other hand, prebiotics have been described as non-digestible feed ingredients for the host with a symbiotic effect that stimulate the growth and activity of beneficial GI bacteria when fed in sufficient amounts (Gibson and Ruberfroid, 1995, Ballou, 2011; Yutaka et al., 2015; Wolfswinkel, 2017). The most commonly used prebiotics in calves are non-digestible carbohydrates (oligosaccharides): mannanoligosaccharides (MOS) and fructooligosaccharides (FOS) (Abney, 2001; Yukata et al., 2015). A prebiotic is characterized by avoiding hydrolysis or

absorption in the upper GI tract. In addition, it might be a substrate that stimulates the growth of at least one beneficial intestinal bacteria or that competes for binding sites, therefore, preventing attachment of pathogens (Gosh and Mehla, 2012; Kara et al., 2015). As a consequence, prebiotics alter the intestinal microflora favoring the beneficial bacteria. Although, as it was mentioned before, there is no consensus on the effects of pre and probiotics on health, performance, and pathogen shedding in newborn dairy calves.

Heat stabilized rice bran is a functional food, classified as a prebiotic, that has shown a wide variety of positive effects on health and immune responses of humans and animals (Henderson et al., 2012; Yang et al., 2014; Goodyear et al., 2015; Sheflin et al., 2015). Rice bran is a by-product of the rice milling process, heat stabilized to prevent rancidity, however, the process does not disturb its bioactivity. Some of the bioactive food components reported in SRB are ɣ-Oryzanol, tocopherols, tocotrienols, polyphenols, phytosterols, carotenoids, aminoacids (tryptophan, histidine, methionine, cysteine, arginine), micronutrients (magnesium, calcium, phosphorus, manganese) and Vitamins (E, B) (Ryan, 2011; Friedman, 2013; Sheflin et al., 2015), which make SRB a potential product to prevent diseases (Ryan, 2011).

Although, rice products have been widely used in animal feeding there is no research focused on the effects of SRB as a possible additive in milk of neonatal dairy calves, as an alternative to the use of antimicrobials.

Published data regarding supplementation of SRB in monogastric animals concluded beneficial effects in prevention of diarrhea and enhancement of immunity (Henderson et al., 2012; Yang et al., 2014; Goodyear et al., 2015). Henderson et al. (2012) reported that a 28 day supplementation of SRB in 4-6 week old mice increased local and systemic IgA by potentiating the activity of *Lactobacillus ssp.*, together with other commensal bacteria. Yang et al. (2014)

reported that a 28 day supplementation plan with SRB in gnobiotic pigs reduced the incidence, the time to recovery, and severity of diarrhea caused by human rotavirus, although, rotavirus shedding was not affected. Intestinal and splenic tissue in conjunction with blood showed an enhancement of the responses of IFN-ɣ, $CD4^{+,}$ and $CD8^{+}$ T cells. Additionally, an increase in the production of IgA and IgM was reported. Furthermore, Goodyear et al. (2015) reported that specific varieties of RB reduced the colonization and fecal shedding of *Salmonella enterica* serovar *tiphymorium* in 4 weeks old mice, in addition to enhancing immunity through the increase of PMN, macrophages, $CD8^{+}$ T cells, and ɣδ cells.

Environmental enrichment and stress

Calves continuously encounter stressors during the first months of life and this represents a significant concern for consumers and the dairy industry. In the last decades, research focused on wellbeing/welfare of calves has grown. Environmental enrichment has been one of the most popular areas of study related to wellbeing in dairy calves and cows. Environmental enrichment includes all of the possible modifications in management or the environment of captive animals that will improve their biological functioning or other validated measures of welfare. In addition, these modifications must be above the accepted minimum management standards (Newby, 1995; Mandel et al., 2016). Furthermore, Mandel et al. (2016) remarked the importance of the 5 categories of environmental enrichment and how each one affects the welfare and behavior of animals in a different way. The categories are social, occupational, physical, sensory, and nutritional environmental enrichment.

Lately, a big emphasis has been made on the impact of pair or group housing on pre-weaned calves. Probably this is the most studied form of environmental enrichment for dairy calves (De Paula Vieira et al., 2012; Costa et al., 2016; Whalin et al., 2018), because a better social and

cognitive development may be achieved when calves are giving the opportunity to have social interaction (Jensen and Kyhn, 2000; De Paula Vieira et al., 2012). Furthermore, calves reared in social isolation have problems coping with stressors, new environments, and situations, in addition to cognitive and social deficits (Costa et al., 2016).

Despite the positives effects on the wellbeing of calves, group housing of pre-weaned calves does not always result in better performance, as is the case for body weight gain (Bolt et al., 2017; Whalin et al., 2018). Additionally, no long term effects of group housing have been analyzed due to the challenge of following animals up until an adult production stage. Studies focused on analyzing the effects in pre-weaning, weaning or after few weeks past weaning provided conclusive results in the improvement of wellbeing. Early social interaction help calves to cope with stress and fearful responses to novel challenges (new environment, commingling, neophofia tests) in the pre-weaned and weaned life (Chua et al., 2002; De Paula Vieira et al., 2012; Costa et al., 2016; ; Bolt et al., 2017; Whalin et al., 2018). Also, learning skills are enhanced in calves reared in pairs, and they showed more flexibility in learning and reversal learning tests (Gaillard et al., 2014). Furthermore, a higher intake of starter was reported in pair housed calves (Whalin et al., 2018).

The type of rearing system has an effect in how calves cope with the stress of weaning and grouping or regrouping. Calves reared in groups, due to their previous social experience, have pre-stablished bonds with some animals and that helps to reduce their stress. Additionally, the early socialization, as it was mentioned before, help weaned calves with their exploratory behavior, cognition (learn where to find food, water, etc.) and social facilitation (Bolt et al. 2017).

Besides pair and group housed calves, there is not abundant information on how to improve welfare of dairy calves through environmental enrichment approach. Automated or mechanical

grooming brushes have recently become commercially available for the use on calves, even though until today there is scarce published data on the potential effects on health, performance, and wellbeing of pre-weaned and weaned calves. Grooming has been described as a low resilience behavior affected by disease or stress and contrary to core behaviors, has a longer recovery phase (Mandel et al., 2017). Adult healthy cattle, constantly use the mechanical or automated grooming devices, but as expected, its use is reduced during disease or stress (Newby et al., 2013; Mandel et al., 2017; Mandel et al., 2018).

Studies conducted with dairy calves have reported a high rate of use of physical enrichment items when they are offered (Georg et al., 2017; Toaff-Rosenstein et al., 2017; Pempek et al., 2017, Zobel et al., 2017). Also, an increase in locomotor play had been reported when pre-weaned calves are housed in hutches with physical items such as brushes (Pempek et al., 2017). Although, grooming activity is considered an innate behavior that could represent a path to improve welfare, there is a lack of information regarding potential effects of grooming devices in health, wellbeing or performance of calves.

Precision dairy farming and calves

Precision dairy farming is a concept that involves the use of different technologies to measure parameters that support and maximize the performance, early detection of disease and production problems of individual animals, minimizing the use of drugs due to prevention and improving the productivity of the farm. The most common parameters measured are physiological, behavioral and production indicators. Some examples of these parameters are heat detection, milk yield, milk conductivity, rumination, activity and laying behavior (Wadsworth et al., 2017).

As covered in the use of brushes for dairy calves, precision dairy farming research has mostly focused on mature cows. However, automated calf feeders have been described as an

important tool for group housing, favoring a more natural feeding behavior and reducing cross suckling (von Keyserlingk et al., 2009; Costa et al., 2016). Additionally, automated feeding systems reduce labor and represent a potential tool to detect sick animals, based on visits to the feeder and level of feed intake (Svensson and Jensen, 2007).

Additionally, current research performed on calves has adopted the use of accelerometer sensors as an alternative to study changes in the behavior of calves under disease, pain or stress (White et al., 2008; Trénel et al., 2009; Hill et al., 2017; Hodson and Timms, 2017). Precision dairy devices provide reliable and objective data, which may be a significant advantage when it is difficult to obtain measurements through direct observation or video recording (Toaff-Rosenstein et al., 2017). Furthermore, changes in behavior, health status and performance are reliable parameters to assess the effectiveness of different management decisions or environment improvements.

Final remarks

Since birth, dairy calves must encounter stressors that might affect health, performance, and survival. Different critical windows must be understood and identified in the rearing period in order to improve wellbeing and health of animals and develop successful management strategies. Nowadays, regulations related to the use of antimicrobials in farm animals are stricter and create a barrier to the type of treatments that might be applied to animals. Therefore, there is a need to study strategies and products to enhance immunity and reduce stress in dairy calves and consequently improve health, wellbeing, and performance of young ruminants. Accordingly, the overriding objective of this thesis is to explore novel strategies to improve health and reduce the stress of pre-weaned and weaned dairy calves.

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CHAPTER 2: STABILIZED RICE BRAN ADDITION IN MILK OF PRE-WEANED ORGANIC HOLSTEIN CALVES

Summary

The first month of life of dairy calves is challenging for dairy producers due their high susceptibility to pathogens and digestive diseases, which may have repercussions on performance and mortality. Our objective was to evaluate the effect of stabilized rice bran (SRB) addition into milk on health, immunity, and performance of pre-weaned dairy calves. Holstein heifer calves (n = 90) were enrolled at 6±1 days old and monitored for 28 days, from July to August, 2017. A paired comparison design with 2 treatment groups was considered. Calves were randomly assigned to a control (CTR; n=45) or a treatment group (RB; n=45) receiving SRB. The RB group received a daily dose of 120g of SRB corresponding to 10% of the daily calories. The SRB dose was divided in 2 feedings (5:00 AM and 7:00 PM) to allow for adequate suspension in milk. A health evaluation was conducted at enrollment and the initial health status was subsequently included as a covariate in the statistical models. Body weight and fecal samples for IgA quantification were performed in the first and last day of the study. Additionally, a daily health evaluation was completed to assess health status and disease severity through diarrhea, dehydration, attitude and milk intake scores. Milk intake was individually recorded after AM and PM feeding. Health status was categorized as healthy, slightly sick, moderately sick, and severely sick. Overall, ADG and IgA concentrations were not affected by the treatment. The total number of calf-days classified as healthy or sick were not different between treatment groups. Similarly, the number of calf-days categorized as slightly affected, moderately sick, or very sick did not differ between treatment groups. The survival analyses indicated no differences in time to first moderate case of disease or in the time to recovery by treatment group and by health status at enrollment.

Our results indicated that the addition of SRB in milk did not have an effect in the health, immunity or performance of pre-weaned dairy calves.

Introduction

Rearing healthy calves and optimizing their growth is essential for the success of dairy operations. However, the first days of life are very challenging; calves might experience the stress of separation of the dam, adaptation to the environment (housing, feeding system, diet, handling) and the development of their immune system.

Diarrhea represents the most important cause of disease and death in the first weeks of life of dairy calves. In 2013, 56.4% of calf mortality was a consequence of diarrhea and animals less than 4 weeks old were the most affected (USDA, 2018). In addition, in 2013, 21% of pre-weaned calves presented diarrhea and 16% were treated with antimicrobials (USDA, 2018). Rehydration, husbandry, and antibiotic therapy are common industry treatments for calves with neonatal diarrhea. However, the regulations for the use of antibiotics in food animals are becoming more restrictive with the years. Consequently, research focused on alternatives to antimicrobials, prevention of diseases, and minimization of environmental risks is needed.

Prebiotic is defined as a non-digestible feed ingredient that stabilizes the intestinal microbiota, stimulating the growth of beneficial bacteria and consequently, inhibiting the colonization by pathogens. This results in an improvement of the immune response (Gibson and Ruberfroid, 1995; Gibson at al., 2005). In contrast, antibiotics eliminate and restrict the growth of detrimental and beneficial microorganisms with no distinction.

The use of prebiotics has been studied in young ruminants as a preventive therapy and as an alternative to antibiotics. The most used products are mannanoligosaccharides, a derivative of the cell wall of the yeast *Saccharomyces cerevisiae.* However, published data is not consistent

with the effects of prebiotics on performance, health and immunity of calves For example, a reduction in the presentation of neonatal diarrhea was reported by Quigley et al. (1997), Gosh and Mehla (2012), Heinrichs at al. (2013), and Froehlich et al. (2017), contrary no differences were found after the supplementation with prebiotics by Abney (2001), Heinrichs et al. (2009), Quezada-Mendoza et al. (2011), Kara et al. (2015). Same situation with weight gain, similar ADG were reported by Froehlich et al. (2017), Kara et al. (2015), and Heinrichs et al. (2009), however a higher ADG were reported by Roodposhti and Dabiri (2012), Ghosh and Mehla (2012) and (Quigley et al., 1997) after the supplementation with prebiotics.

Heat stabilized rice bran (SRB) is a prebiotic that has not been studied in young ruminants and its potential as a supplement or additive in whole milk of dairy calves has not been explored. However, research in other species and humans conclude that SRB has effects preventing diseases, promoting the growth of beneficial intestinal microorganisms, and stimulating immune responses (Sierra et al., 2005; Henderson et al., 2012; Friedman, 2013; Yang et al., 2014; Sheflin et al., 2015).

We hypothesized that the addition of SRB in milk of pre-weaned calves would reduce the presentation and severity of neonatal diarrhea, improving the immune response and consequently the overall performance. Therefore, our specific objective was to determine the effect of SRB on average daily gain (ADG), IgA concentration, presentation of diseases, time to recovery from disease and, animal removal.

Materials and Methods

This study was conducted in a certified organic dairy calf rearing facility located in Northern Colorado. Pre-weaned dairy calves were managed during the study in accordance to the guidelines set by the Institutional Animal Care and Use Committee of Colorado State University (Protocol ID: 16-6893A).

Animals, housing and feeding

Ninety pre weaned Holstein heifer calves, 6± 1 days old, were enrolled in this research. Calves were monitored during 28 days to assess the effect of Stabilized Rice Bran (SRB) addition in milk. After the SRB plan was finalized, a follow up period until weaning was completed to evaluate health outcomes based on farm records. The first stage of the study began in July 2017 and ended in August 2017. The second stage was completed in October 2017.

All calves were immediately separated from their dam at birth, fed 2.8 L of colostrum during the first hour of life and had their navel disinfected with a 7% iodine tincture solution. Colostrum feeding was repeated at 3 and 8 hours of life. The quality of colostrum was assessed in the maternity facility with a Colostrometer^{TM} (Biogenics, Florence, FL) and contained at least 50 mg/ml IgG. After transferring calves to the rearing area, they received 3.8 L of colostrum in intervals of 10 hours for 4 times.

After arrival in the rearing facility, calves were housed in rows of 90 individual hutches (Agri-Plastics, Stoney Creek, ON, Canada) with sand bedding and a wire panel pen attachment of 2.25 m^2. Calves had visual but no physical contact with other animals until weaning.

Milk was provided in 2.8 L bottles (E-Z Nurse^{TM}) three times a day. During the period of study the feeding schedules were 5 AM, 12 PM, and 7:30 PM. Pasteurization and preparation of the delivery trailers started 1 hour before the specified feeding time.

Milk collected from the hospital pen, and organic fluid milk delivered each day from an organic processing plant was pasteurized. Also, organic powder milk was provided, following preparation instructions.

Milk bottles were collected, cleaned, disinfected, and prepared for feeding in two trailers with a capacity of 500 bottles each. Milk was served into the bottles through a pipeline system and

delivered to the calves attaching the trailers to tractors. Milk temperature ranged between 105°F and 112 °F depending on weather conditions.

Organic certified calf starter was offered to the calves from day 4 of life in small amounts in clean buckets (16% Organic Calf Starter, Feedex Companies, LLC. South Hutchimsin, KS, A2.1). Water was offered *ad libitum* since the arrival of calves. Clean water was provided 3 times per day, and grain was checked and filled two times a day (morning and night) depending on calf consumption. Two shifts per day ensured a constant flow of water and grain to all the calves in the facility.

The farm vaccination program included the administration of Inforce-3 (Bovine Rhinotracheitis, Parainfluenza 3, Bovine Respiratory Syncytial Virus. Zoetis Inc.) at day 1 of life. Ultrabac 8 (*Clostridium chauvoei, Cl. Septicum, Cl. Haemolyticum, Cl. Novyi, CI. Sordellii, Cl. perfringens* Types C and D. Zoetis Inc., Florham Park, NJ) was administered at 21 days of life. Spirovac L5 (*Leptospira canicola, L. grippotyphosa, L. icterohaemorrhagiae* and *L. Pomona, L. borgpetersenii* serovar hardjo type hardjo-bovis. Zoetis Inc., Florham Park, NJ) and a booster of Inforce and Ultrabac 8 were administered at 42 days of life.

Total proteins were measured by trained personal to evaluate passive transfer of immunity. A 5 ml blood sample was collected from calves 3 to 9 days old in a tube without anticoagulant. The sample was allowed to clot before centrifugation. Serum was analyzed in an optical engine digital refractometer (Palm Abbe^{TM,} Solon, OH) and all readings were kept in the farm recording system.

Dehorning was performed by trained personnel before 30 days of age, using local anesthesia with 2% lidocaine followed by electrical hot iron cauterization and administration of anti-inflammatories. Procedures were performed under veterinarian supervision.

The completion of the step down weaning process took three weeks and it was based on calf starter consumption (1.8 to 2.2 kg per day). During the first week of weaning the night feeding was suspended. During the second week, the noon feeding was suspended. In the last week, the morning milk feeding was suspended. All weaned calves stayed during the third week in the individual hutches to monitor stress and health before transferring to collective pens.

Trained personnel had the responsibility to perform daily health evaluations to all the calves in the facility, with the objective of detecting and monitoring sick animals to apply treatments stablished in the farm SOP.

Experimental design and treatment groups

A paired comparison design with 2 treatment groups was performed. Calves were randomly assigned to a control (CTR, n=45) or a treatment group (RB, n=45) and identified with different color clips in the farm ear tag ID. Two purple clips were used per each RB calf. Two white clips for CTR calves, due to management and feeding routine a blinded study was not possible. At enrollment, a clinical examination was completed to determine the health status of each calf.

All calves enrolled were weighted using a mobile platform digital scale (Caf-Cart. Raytec LLC, Ephrata, PA) at enrollment and at day 28. This procedure was performed after the morning feeding.

A subsample of 10 calves from each group was randomly selected to collect fecal samples at enrollment and at day 28 of the study, after the morning feeding. Twenty grams of fecal matter were obtained by rectal stimulation with a gloved finger and stored in two separate sterile containers. One set of samples was submitted fresh to Colorado State University, Veterinary Diagnostic Laboratories for Coronavirus and Rotavirus screening. The second sample was frozen at -20°C for subsequent IgA analysis (IgA Bovine ELISA kit, Abnova Corporation, Taipei,

Taiwan.). For IgA quantification, the first step was a dilution of 30 mg of fecal matter in 300 µl of deionized water. Secondly, 5 µl of the supernatant was diluted in 495 µl of the diluent solution. Finally, 30 µl of the second dilution was diluted in 270 µl of diluent solution to obtain a 1/1,000 dilution as recommended by the manufacturer.

A daily health assessment was performed for each calf every morning after the milk feeding. The calf health scoring chart by University of Wisconsin (McGuirk, 2008) was modified to assess fecal score. The scoring was categorized as normal or 1 for score 1 and 2 in the chart, as abnormal or 2 for score 3 in the chart and as severe or 3 for score 4 in the chart.

Dehydration status was assessed daily using a calf dehydration chart (Wattiaux, 2005; A2.2, A2.3). The scores were assigned as 1 for non-dehydrated animals (<6% water body loss) with a good attitude, strong suckle reflex, appetite, no eyeball retraction into the orbit and skin tent lower than 2 seconds. Score 2 was described as moderate dehydration (6 to 8% of water body loss) were the calf was depressed with weak suckle reflex, dropped ears, dry and slightly recessed eyes into the orbit and skin tent duration of 2 to 6 seconds. Score 3 was described as severe dehydration (>8% of water body loss), when the calf showed signs of depression no suckle reflex, skin tent >6 seconds, dry and recessed eyes into the orbit and recumbency.

Attitude was recorded daily in conjunction with the health assessment. A depression scoring system to determine sickness (Perino and Apley, 1998; Coetzee, 2012) was modified defining 3 attitude outcomes. Outcome 1 corresponded at score 0 from the chart; outcome 2 corresponded at scores 1 and 2 from the original chart; outcome 3 corresponded to scores 3 and 4 from the chart (Table 2.1).

Milk intake was recorded after the AM and PM feedings for all the calves that participated in the study. The intake was divided in 5 categories, depending on the milk refusal (0%; 25%; 50%; 75%; 100%). Subsequently, an average daily intake was calculated.

Each animal was assigned with a daily health severity score, based on the combined morning health assessment (diarrhea score, dehydration score, attitude score) and the average milk intake. A status of "healthy" was determined when all the scores were 1 (normal) and a milk refusal ≤25%. A "slight" disease status was applied to all the calves that had a milk refusal below 50% and one health score of 2. In the case of diarrhea, a score 3 was also considered "slight" when the calf was not dehydrated and its attitude was not compromised. A "moderate" disease status was applied to the calves that presented more than two health scores of 2 (or diarrhea score 2 or 3) and milk refusal above 50%. A "severe" disease status sick was given to calves in recumbency with more than two health scores in 3 and milk refusal above 75%.

Ambient temperature and relative humidity were measured using a HOBO Pro-v2 logger (Onset computer Corporation, Bourne, MA). Daily Temperature Humidity Index (THI) was calculated using the formula, THI= (1.8 x Temperature + 32)-((0.55-0.0055 x Relative Humidity) x (1.8 x T -26)) (Kendall et al., 2008; Vickers et al., 2010; Manriquez et al., 2017). Individual total protein values from each calf were obtained from farm records.

Feeding

Organic Jasmine Stabilized Rice Bran provided by Rice Bran Technologies, Sacramento, CA (Table 2.2) was stored in 50 lb sealed bags and used in the milk feeding addition plan. The bags were kept in a covered shed protected from weather conditions.

The dose of SRB was calculated to add 10% of the daily calorie intake during the first weeks of life (400 calories), based on research with animal models (Henderson et al., 2012; Yang

et al., 2014; Goodyear et al., 2015). The daily dose of SRB was mixed in the milk of the morning and the night feedings, as a higher intake was observed at these times compared with noon feeding.

To obtain a homogenous SRB distribution, the rice bran was placed in the bottles before the milk was added. Immediately after the milk was placed in the bottles, the content was blended with a whisker and a drill.

When the milk temperature ranged between 39 - 41° C the bottles with SRB were taken from the feeding trailer and served by hand in a utility task vehicle. Control calves were fed simultaneously by farm personnel. Immediately after calves finished milk consumption, AM and PM intake was recorded and the bottles were collected for cleaning and sanitization, following the standard operating procedures (SOP) of the farm. The procedures were similar in the morning and night feeding.

Statistical Analysis

Data were analyzed using SAS statistical software (9.4, SAS Institute Inc., Cary, NC). Calf was considered the experimental unit of analyses. Treatment group and health status at enrollment were included in the models unless otherwise specified.

Based on their distribution, serum total protein (TP) measurements were categorized in two levels to detect failure in passive immune transfer or dehydration: < 7.5 g/dL and ≥ 7.5 g/dL. A simple logistic regression was used to analyze differences in total serum protein measurements between treatment groups (PROC LOGISTIC).

Health status at enrollment was categorized as “healthy” or “diseased” and group differences were analyzed using simple logistic regression (PROC LOGISTIC). This analysis was performed to assess the initial health condition between the individuals of the two treatment groups.

The association between days sick (categorized by severity of disease) and treatment group was initially analyzed by use of Chi square test (PROC FREQ). Subsequently, a repeated measures analysis for a binary response (PROC GENMOD), assuming an exchangeable correlation structure was performed. Calf was considered the subject in the repeated statement. Total calf days "healthy" were compared with total days "sick" (combining "slight", "moderate" and "severe"). Conversely, total days calves spent in the "severe" category were compared with the other categories ("healthy", "slight" and "moderate"). In addition, total days calves spent with a "slight" disease condition were compared with the combination of "moderate" and "severe" days. Finally, days in "severe" condition were compared combining days with "slight" and "moderate" condition.

Time to event analysis (PROC LIFETEST) was used to evaluate differences in time to presentation of the first "moderate" case of disease between the 2 groups. Also, time to event analysis was used to evaluate the time to recovery from a "moderate" status to a "healthy" or "slight" status (lasting at least 3 days). The Wilcoxon test was used to determine significant differences. In addition, the means for recovery time by group were calculated using PROC MEANS.

The association between milk intake and THI, was assessed by repeated measure analysis ANOVA (PROC MIXED). The model included treatment group and THI by day in study and the interaction between treatment group and THI by day.

Least square means (LSM) by group were calculated for initial weight, final weight, gain weight and ADG using PROC GLM. To determine differences in the concentration of IgA by treatment group at the end of the addition plan, a normalization of the data was performed through a Log10 transformation.

Subsequently, least square means (LSM) were calculated using PROC GLM. The model included group, health at enrollment, and IgA concentration at enrollment as a covariate.

PROC FREQ was used to determine the frequency of the animal removal (mortality and culling) between groups. Moreover, group differences in mortality and culling in the study period and in the follow-up period were analyzed using logistic regression (PROC LOGISTIC). Both events were aggregated in one variable for the analysis.

Additionally, the Kaplan Meier survival analysis (PROC LIFETEST) was used to evaluate differences in the time calves left the study due to death or culling. Both events were aggregated in one variable. The Wilcoxon test was used to determine significant differences.

Logistic regression was used to analyze differences in the presentation of diseases (<2 vs ≥2 or more diseases) within the follow-up period (PROC LOGISTIC). In addition, a survival analysis (Kaplan Meier/PROC LIFETEST) was used to evaluate differences in time to first disease after the end of the addition of SRB. The Wilcoxon test was used to determined statistical significance. Statistical significance was defined at $P < 0.05$. Tendency was defined at $0.05 < P < 0.1$.

Results

Overall, 88 calves were considered for the analyses. Two RB calves were excluded because they rejected the milk in the first 2 days of SRB addition. Thirty one calves presented TP measurements above 7.5 g/dL, indicating that 35% of the enrolled animals presented some degree of dehydration (Table 2.3). No difference (P=0.94) was found in the odds of presenting TP above 7.5 g/dL (OR= 1.03, 95% CI= 0.43-2.47) for CTR calves in comparison with RB calves. Additionally, all calves presented TP measurements above 5.5 g/dL indicating no failure in passive immune transfer. At enrollment, 43 calves presented signs of slight disease (diarrhea), representing

49% of the animals (Table 2.4) explaining the potential dehydration found in the TP measurements. No differences (P=0.39) were found in the odds of disease at enrollment (OR=1.44, 95% CI= 0.62-3.34) for the CTR in comparison with RB group.

In total, calf-days "healthy" and calf-days "sick" were 1,198 and 1,230 respectively. These cumulative days were categorized by disease severity and treatment group (Table 2.5). The repeated measure analyses, did not indicate significant associations between the occurrence of "healthy" or "sick" days ("slight", "moderate" or "severe") for any of the comparison that were made between treatment groups and disease categories (Table 2.6)

No differences were found in the time to first "moderate" disease status between treatment groups (P=0.71), although, the survival curve demonstrated a pronounced slope (Figure 2.1a) during the first 5 days. About 70% of RB calves and 60% of CTR calves presented the first "moderate" disease status in the first 5 days of the study. At day 10 of the study, 80% of all calves already presented a "moderate" status of disease. A tendency was found in the survival function when health at the enrollment was added as a covariate (P=0.08). Mean days for the presentation of a "moderate" status indicate that sick calves at enrollment presented this status earlier than enrolled healthy calves (Table 2.7). At day 5 more than 70% of sick RB calves presented a "moderate" status for the first time (Figure 2.1b). At day 5, around 40% of healthy CTR calves presented this condition (Figure 2.1b).

Regarding time to recovery from a "moderate" status to a "slight" or "healthy" status, a tendency for a difference between treatment groups was found in the Kaplan Meier analysis (P=0.052). Control calves reduced the severity of disease to a "slight" status in a shorter period of time than RB calves (Figure 2.2a). Means for time to recovery indicated that CTR calves recovered from a "moderate" status in 3.1 days and RB calves in 4.9 days (Table 2.8). When health at

enrollment was added as a covariate, no differences were found in the time to recovery (P=0.12). Despite of these results, the survival curve considering health at enrollment indicated that "healthy" CTR calves at enrollment recovered faster than the other groups. "Sick" RB calves at enrollment recovered slower than the other groups (Figure 2.2b). Means of recovery for "healthy" CTR calves were 2.8 days and means of recovery for "sick" RB calves were 5.7 days (Table 2.8).

The repeated measures analysis did not indicate significance for the interaction between daily intake and daily THI (P= 0.76) for treatment groups. Main effect day (P<0.0001) demonstrated differences in the intake by day in study (Figure 2.3). Main effect treatment group was not significant (P= 0.08). There was an increase in milk intake with time, increasing from around 70% to 100% at the end of the study. During the first 15 days, RB calves intake was higher than CTR calves (Figure 2.3).

The results provided by The Veterinary Diagnostic Laboratories at Colorado State University, on fecal samples for the detection of Coronavirus and Rotavirus indicated that all the selected calves (n=21) were negative for Coronavirus in the two sampling dates. In the first sampling 8 calves (CTR=2, RB=6) were positive to Rotavirus. All calves were negative to Rotavirus in the subsequent sampling.

No differences were found for ADG between treatment groups, for the 28 day of study (P=0.47, Table 2.9). The concentration of IgA did not differ between treatment groups and health status at enrollment after the addition of SRB (P=0.17). The mean value (SE) for CON was 3.795 ± (0.10) ng/ml and for RB was 3.541 ± (0.12) ng/ml. However, a decrease was observed in the concentration of IgA for both treatment groups from sampling at enrollment to sampling at the end of the addition plan (Table 2.10).

Overall, 9 out 90 calves enrolled died (CRT= 4, RB=2) or were culled (CTR=2, RB=1). Therefore, 6 CTR calves and 3 RB calves were lost during the 28 day of study. The odds of leaving the study due to death or culling (OR=1.93, 95% CI= 0.44-8.26) comparing CTR group with RB group did not differ (P=0.37). Additionally, no differences were found in the time calves left the study due to death or culling (P=0.29, Figure 2.4a).

Once the addition of SRB finished, a follow up period until weaning was performed through the use of health records provided by the farm. Seventy nine calves completed the 28 days of SRB addition, 25 (CTR=11, RB=14) of these animals received organic treatment for at least one disease event during the follow up period. Ten calves (CTR=5, RB=5) presented more than 1 event of disease between d28 in study and weaning. No differences (P=0.92) were found in the odds of presenting more than 2 events of disease between treatment groups using health at enrollment in the model (OR=0.93, 95% CI= 0.08-1.38). In addition, time to a first disease event after finishing the study period did not differ between treatment groups (P=0.43, Figure 2.5), the main reason for disease was respiratory problems.

In total, 16 out of 79 calves were lost during the follow up period. Eleven calves were sold (CTR=5, RB=6) and 5 calves died (CTR=2, RB=3). No differences were found in odds to left the study by treatment group (P= 0.63, OR=0.76, 95% CI= 0.25-2.31). Additionally, time to death or culling did not differ between groups (P=0.63, Figure 2.4b)

Discussion

The use of SRB or rice-based products has not been extensively studied on pre-weaned ruminants. Rice bran in other animal species and humans showed positive effects in reducing presentation and duration of diarrhea, increasing the production of IgA and enhancing the immune system (Henderson et al. 2011; Ryan, 2011; Borrensen and Ryan, 2014; Yang et al., 2014;

Goodyear et al., 2015). On the other hand, published studies regarding the use of prebiotics are not conclusive in the effects on pre-weaned calves. Therefore, the objective of this project was to evaluate the effects of SRB addition on performance and health of newborn calves.

Total serum protein (TP) in calves is a valuable tool to measure passive immune transfer and consequently new born and colostrum management practices at farms. Overall, all the enrolled calves had TP measurements above 5.5 g/dL. It has been described that concentrations ≥5.2 g/dL in healthy calves and ≥5.5g/dL in clinically ill calves is considered a measure of adequate passive transfer of immunity (Weaver et al., 2000; McGuirk and Collins, 2004). However, the concentration of total serum protein in calves might be affected by dehydration. In general terms, refractometer readings with concentrations in a range between 5.5g/dL and 7.5g/dL have been recommended as parameter of successful passive immune transfer at farms (Donovan et al., 1998; Ferguson, 2018).

At enrollment, 35% of our animals presented TP concentrations above 7.5g/dL and almost 50% showed signs of clinical disease (diarrhea or slight dehydration). However, no differences were found between treatment groups, indicating that our groups started in a similar immune and health condition. The beginning of our study coincided with nutritional management adjustments made by the farm that resulted in an increase in the incidence of neonatal diarrhea, therefore, health at enrollment was assessed and added in our models.

Overall, our results did not report a difference in the number of days calves were sick or differences in disease severity for treatment groups. However, RB calves spent in total more days "healthy" but more days "moderate" and "severe" sick in comparison with CTR group.

Sweeney (2000) reported that the use of an oral electrolyte containing rice, promoted diarrhea in young calves less than 2 weeks old. Pre ruminant calves lack the production of enzymes

to digest maltose and starch from rice and this situation might lead to osmotic diarrhea when it is provided in milk replacers or in oral electrolytes (Dollar and Porter, 1957; Sweeney, 2000). This fact might explain the increase in the days calves spent moderate and severely sick in our study. In our knowledge, no previous studies have been performed in pre-weaned ruminants using SRB as prebiotic added in whole milk. However, rice protein has been used as a replacement of whey protein in milk replacers and the results were not positive in terms of performance of calves (Hill et al., 2008). However, in that study health parameters were not collected or reported

Published studies using prebiotics as a prophylactic or treatment therapy in pre-weaned calves are limited and there is not a consensus on the effects of prebiotics on health and diarrhea presentation in young dairy calves. Positive effects were reported in the reduction of disease presentation or diarrhea scores (Quigley et al., 1997; Ghosh and Mehla, 2012; Heinrichs et al., 2013; Froehlich et al., 2017). However, other studies did not find significant differences (Quezada-Mendoza et al., 2011; Kara et al., 2015). Additionally, studies not reporting differences observed a greater percentage of control calves with more cases of diarrhea than calves treated with prebiotics (Abney, 2001; Heinrichs et al., 2009). Some of these researches worked with calves in excellent health conditions making difficult to describe the real potential of the treatments (Heinrichs et al., 2009).

No negative effects for the use of prebiotics had been reported. In our case, we started with almost 50% of the animals presenting some degree of diarrhea or dehydration. Therefore, the addition of certified organic SRB was tested in a challenging population and no significant effects were observed on the total number of sick days in our calves. Santos et al. (2015) described a similar situation with the addition of essential oils (EO) in milk of pre-weaned calves. No differences were found in presentation or scores of diarrhea, but treatment calves presented more

severe diarrhea scores. Authors explain that EO might have adverse effects in the intestinal tract of young animals by reducing enzyme activity, changing the physiology and anatomy of the intestinal epithelium.

The decision of analyzing the time to a first "moderate" status of disease was made considering the health situation at enrollment. Calves presented a first "moderate" health condition as result of diarrhea in the first 5 days in study, when they were 10 to 12 days old. The typical pattern for presentation of neonatal diarrhea in calves starts and peaks between week 2 and week 3 of life and decline and stabilize around week 5. In comparison with published data, our calves were challenged early in life (Heinrichs et al., 2009; Santos et al., 2015). Despite of this situation, treatment groups did not differ in the time to a first "moderate" status. As it was expected, calves that were sick at enrollment showed a tendency to present the first "moderate" health status before than healthy calves at enrollment. Contrary to our expectations, RB calves that were healthy at enrollment presented a moderate health status before than healthy CTR calves. Moreover, RB calves sick at enrollment were the first in presenting a "moderate" disease status in the survival curve. Even though this results were not significant, we attribute this findings to a possible osmotic effect of SRB on the large intestine of young animals (Sweeney, 2000). Our results contrast with neonatal animal model research, where SRB had a protective effect in the presentation of disease through the stimulation of the immune response and increase of probiotic bacteria in the gastrointestinal tract (Yang et al., 2014).

Even though, no statistical differences were found in the time to recovery from a "moderate" to a "slight" health status between treatment groups and health at enrollment, RB calves had a larger mean time for recovery than CTR calves (RB=4.9 vs CTR=3.12 days). RB calves that were sick at enrollment recovered in 5.7 days, in comparison, CTR calves sick at

enrollment recovered in 3.38 days. This information is valuable suggesting that SRB may potentially have a detrimental effect in the time neonatal calves recovered from a diarrhea episode, probably explained in the incapacity to digest carbohydrates and starches from rice (Sweeney, 2000). Other studies using prebiotic or EO did not reported differences in days sick or days in treatment for supplemented calves (Santos et al., 2015; Kara et al., 2015).

In our study, we observed a progressive increase in milk intake in both groups, related to an age effect rather than to treatment or THI. During the first 7 days RB calves had higher intakes. This might have occurred due to the consistence in the flavor of milk and palatability of SRB. Only 2 calves out of 45 rejected SRB milk since day 1. One difficulty that we observed was the necessity of an intense mixing to suspend the SRB dose in milk. Furthermore, if milk was not served soon after mixing, SRB started to decant in the bottom of the bottle. Frequently, we observed residues and clumps of SBR in the nipple and less frequently in the walls of the bottles. This accumulation did not block the teats and most of the calves continued suckling the SRB. With the pass of the days and as the calves get a stronger suckling reflex the amount of residues decreased and the bottles were cleaner. A similar situation related with the presence of small clumps in the nipples, despite of a thoroughly mixed was reported by Quingley et al. (1997). Our daily dose of SRB was larger than that of published studies testing prebiotics on pre-weaned calves, where authors worked with commercial products in doses no greater than 7 g/d (Heinrichs et al., 2009; Quezada-Mendoza et al., 2011; Ghosh and Mehla, 2012; Roodposhti and Dabiri, 2012; Kara et al., 2015). We offered SRB in its natural form in a dose of 120g/d (only heat stabilized to prevent rancidity).

Presence of fecal rotavirus and coronavirus were analyzed in samples collected at enrollment and at the end of the study period. With exception of a few animals in the first sampling, these viruses were not found in CTR or RB calves. SRB has been linked with a protective response

against diarrhea promoted by rotavirus in gnotobiotic pigs fed with dietary RB (Yang et al., 2014) reducing the severity of the diarrhea but not the shedding of rotavirus. Published data also confirmed an effect of RB preventing the colonization of *Salmonella spp* and regulating the intestinal immunity in mice fed with dietary RB in the same percentage as our study (Goodyear et al., 2015). Dietary RB had been linked with an increased colonization of *Lactobacillus spp* in nearly 500% (Henderson et al., 2012).

The microbiota of the calves were not analyzed. Some studies using prebiotics or EO in dairy calves have measured the effect on bacteria populations. However, published data are not consistent. Some studies did not report an effect of prebiotic supplementation on relation of beneficial and pathogenic bacteria in feces (Heinrichs et al., 2009; Kara et al., 2015; Santos et al., 2015). Quezada-Mendoza et al. (2011) reported an increase in beneficial bacteria (*Lactobacilli* and *Bifidobacteria)* in the first days of life but it was not related with a reduction in coliform bacteria shedding. In addition, Roodposhti and Dabiri (2012) reported a reduction in *E.coli* shedding.

Control calves and RB calves did not differ in ADG during the 28 days that RB was added to the milk. Published data is not consistent with differences in ADG in calves fed with prebiotics until weaning. No differences were reported by Froehlich et al. (2017), Kara et al. (2015), and Heinrichs et al. (2009). The same situation was observed in research with EO. ADG was not affected for calves fed with different concentration of EO mixed in milk from birth to weaning (Santos et al., 2015). Conversely, Roodposhti and Dabiri (2012) found a greater ADG in pre-weaned calves fed with prebiotic (MOS) in milk for 60 days in comparison with control calves. Ghosh and Mehla (2012) reported positive differences in the ADG of calves fed with 4 g of prebiotic (MOS) in milk for 60 days in comparison with control group (322 vs 263 g/day). In

addition, a supplementation plan with galactosyl-lactose for 26 days added in the milk of pre-weaned calves, reported an increased ADG of 125 vs 197 g/day (Quigley et al., 1997). Interestingly, a study that used rice protein to replace 25 to 50% of whey protein in milk replacers found that body weight of calves less than 2 month old reduced between 12 to 54% in comparison to control calves. It was stated that this replacement and the poor ADG can be due to an inability of the neonatal calf to efficiently digest the crude protein and amino acids in rice protein concentrate (Hill et al., 2008; Sweeney, 2000).

Our study did not find differences in IgA concentration in feces between treatment groups but concordantly with published data it was observed a reduction of the concentration of IgA related with an increase in the age of the calves (Heinrichs et al., 2009). At enrollment IgA quantification was the higher probably due to the presence of colostrum antibodies.

Published data is not consistent about the immunomodulatory response of prebiotic fed to pre-weaned calves and the quantification of fecal IgA. Quezada-Mendoza et al. (2011) did not find an effect of a commercial prebiotic (Prebio Support, Meiji Feed Co., Ltd. Tokyo, Japan) in fecal and salivary IgA of calves fed with milk replacer. Conversely, Heinrichs et al. (2009), using the same prebiotic reported a higher count of IgA in supplemented calves at week 2 and 4 (Prebio Support was fed until weaning). Another study reported a higher concentration of IgA from day 9 to 20 of life, time that coincided with a reduced presentation of diarrhea when calves were supplemented with mannanoligosaccharides (MOS) (Heinrichs et al.; 2013).

The immunomodulatory response of dietary RB has been described in animal models. Specifically related with the production of IgA, Henderson et al. (2012) reported an increased production of mucosal IgA in 4 to 6 weeks old mice fed 10% of the daily calories for 28 days. Similar results were found by Yang et al. (2014) reporting an increase in the serum titer of IgA in

gnotobiotic pigs. Rice Bran enhances the growth of *Lactobacillus ssp.* which might be one of the potential mechanism of the immune modulation. Although, Henderson et al. (2012) found a low Lactobacillus-specific IgA in their study but an increased IgA production. They postulate that RB induces the modulation of different beneficial bacteria that might increase the IgA concentration in intestine.

In our study no differences were found in the odds of leaving the study due to death or culling by treatment group. Although, in the 28 days of SRB addition, the double of CTR calves left the study in comparison with the RB group (CTR=6 vs RB=3). Conversely to what was expected, the follow up period until weaning indicated that the same number of animals were lost in each group (CTR=13 vs RB=12), the main reason for removal in this period was respiratory problem. Overall, SRB did not had an effect on the proportion of animals removed from the farm from birth to weaning. Congruently with our results, Abney (2001) did not describe differences in mortality of calves fed with prebiotic in comparison with control calves. Additionally, no differences were found in the cases of disease detected by farm personnel from the end of the supplementation until weaning. The main cause of treatment was respiratory disease, followed by diarrhea. Overall, 10 calves were treated for more than 1 disease episodes (CTR=5 vs RB=5). Consequently, the 28 days of addition of SRB in the milk of calves did not have influence in health outcomes in the pre-weaning.

Therefore, the addition of SRB in the diet of neonatal calves is discouraged due to a possible malabsorption syndrome that can accentuate diarrhea episodes.

Conclusions

Contrary to previous research in monogastric animals, our findings suggest that the addition of SRB in the milk of newborn calves for 28 days did not enhance performance, health or

immunity during the first month of life, a period characterized for the presentation of digestive diseases. Furthermore, no differences were found from birth to weaning in the presentation of diseases or removal of animals. Further research is encouraged in older calves to investigate the potential beneficial effects of SRB in their performance, health, and immunity.

Tables and Figures

Table 2.1: Depression scoring system to determine disease (Perino and Apley, 1998, Coetzee, 2012)

Score	Clinical Signs
0	Normal, no signs of depression.
1	Noticeable depression without apparent signs of weakness. Slower than other calves but actively follows movements with raised head.
2	Marked depression with moderate signs of weakness without a significant altered gait. Stands with head lowered. Calf will perk up when approaches but will return to depress stance, moves slowly and may display incoordination.
3	Severe depression with signs of weakness such as a significant altered gait. Obviously weak; raises head just when approached closely.
4	Moribund, unable to rise.

Table 2.2: Nutritional composition of the organic SRB fed to pre-weaned calves. Guaranteed analysis provided by manufacturer (Rice Bran Technologies, Sacramento, CA).

Nutrient	Concentration
Energy (kcal/100g)	330.5
Protein (g/100g)	14
Fat (g/100g)	21
Total carbohydrate (g/100g)	49
Available carbohydrate (g/100g)	24
Crude Fiber (g/100g)	9.44
Soluble dietary fiber (g/100g)	2.82
Free fatty acids (g/100g)	1.34
Choline (mg/100g)	178
Vitamin B3 (mg/100g)	36.7
Vitamin B5 (mg/100g)	3.26
Vitamin B6 (mg/100g)	5.15
Cu (mg/100g)	0.64
Mg (mg/100g)	785
Mn (mg/100g)	11.4
P (mg/100g)	2094
K (mg/100g)	1112
Zn (mg/100g)	4.84
Alpha Tocopherol (mg/100g)	5.91
Beta Tocopherol (mg/100g)	0.76
Gamma Tocopherol (mg/100g)	1.24
Biotin (µg/100g)	25.6
Vitamin B12 (µg/100g)	0.38
Cr (ppm)	0.26

Table 2.3: Frequency of animals by serum total protein (TP) category, stratified by treatment group (P=0.94)

	Group		
TP reading (g/dL)	CTR	RB	Total
5.5-7.4, n (%)	29 (33.0)	28 (31.8)	57 (64.8)
≥7.5, n (%)	16 (18.2)	15 (17.0)	31 (35.2)
Total, n (%)	45 (51.2)	43 (48.8)	88 (100)

Table 2.4: Frequency of animals by health status at enrollment and treatment group (P=0.39).

	Group		
Health at enrollment	CTR	RB	Total
Healthy, n (%)	21 (23.8)	24 (27.3)	45 (51.1)
Sick, n (%)	24 (27.3)	19 (21.6)	43 (48.9)
Total, n (%)	45 (51.1)	43 (48.9)	88 (100)

Table 2.5: Cumulative days spent by calves in the 4 different disease categories during the 28 days of SRB addition. No difference was determined in the total days spent "healthy" or "sick" (P=0.43).

	Group		
Disease status	CTR	RB	Total
Healthy, n (%)	569 (23.4)	629 (25.9)	1198 (49.3)
Slight, n (%)	481 (19.8)	429 (17.7)	910 (37.5)
Moderate, n (%)	130 (5.4)	168 (6.9)	298 (12.3)
Severe, n (%)	8 (0.3)	14 (0.6)	22 (0.9)
Total, n (%)	1188 (48.9)	1240 (51.1)	2428 (100)

Table 2.6: Odds ratios for daily disease severity status for control calves compared with rice bran supplemented calves.

Cumulative days categorized by disease severity	Odds Ratio	95% CI	*P-value*
Healthy vs Sick	1.13	0.82-1.57	0.43
Severe vs healthy, slight, moderate	0.65	0.16-2.58	0.54
Slight vs moderate and severe	0.77	0.52-1.13	0.19
Severe vs slight and moderate	0.58	0.15-2.22	0.43

Table 2.7: Mean and median days for the presentation of a first "moderate" disease status by health at enrollment and treatment group (P=0.08).

Group	Health at enrollment	N	Mean for first moderate status (days)	Median for first moderate status (days)
CTR	Healthy	21	9.09	5
	Sick	24	6.62	3.5
	Overall	45	7.77	4
RB	Healthy	24	8.62	4
	Sick	18	7.73	2
	Overall	42	8.23	4

Table 2.8: Mean and median days for the time to recovery from a "moderate" disease status to a "slight" disease status kept for at least 3 consecutive days by enrollment and treatment groups (P=0.12).

Group	Health at Enrollment	N	Mean for recovery (days)	Median for recovery (days)
CTR	Healthy	19	2.84	1
	Sick	21	3.38	3
	Overall	40	3.12	3
RB	Healthy	20	4.3	4
	Sick	15	5.73	4
	Overall	35	4.91	4

Table 2.9: Least square means and standard error for initial weight, final weight, gain weight and ADG for treatment groups.

	Group		
Variable	CTR	RB	P-Value
Initial Weight (Kg)	39.6 ± 0.81	38.5 ± 0.82	0.32
Final Weight (Kg)	54.1 ± 1.03	54.3 ± 1.02	0.89
Gain Weight (Kg)	14.9 ± 0.86	15.8 ± 0.85	0.47
ADG (Kg/d)	0.53 ± 0.03	0.56 ± 0.03	0.47

Table 2.10: Fecal IgA concentrations (ng/ml) at enrollment and at the end of the addition plan with rice bran by treatment group and health at enrollment (P=0.17)[1].

Group	Health at enrollment	ID	Initial IgA (ng/ml)	Final IgA (ng/ml)
CON	Healthy	84026	4.00	3.80
		84066	3.97	4.00
		84082	3.91	3.78
	Sick	84024	4.02	3.50
		84030	3.64	3.55
		84032	3.87	3.99
		84073	4.03	3.05
		84102	3.86	4.10
		84106	3.80	3.66
		84108	3.76	3.89
RB	Healthy	84033	3.51	3.58
		84035	3.99	3.13
		84077	3.98	3.67
		84081	3.85	3.63
		84083	4.01	3.73
		84085	3.75	3.97
		84097	3.72	3.68
	Sick	84025	3.73	3.98
		84075	3.78	3.28
		84095	3.27	2.83

[1]P-value for the difference of IgA concentration by treatment group and health at enrollment.

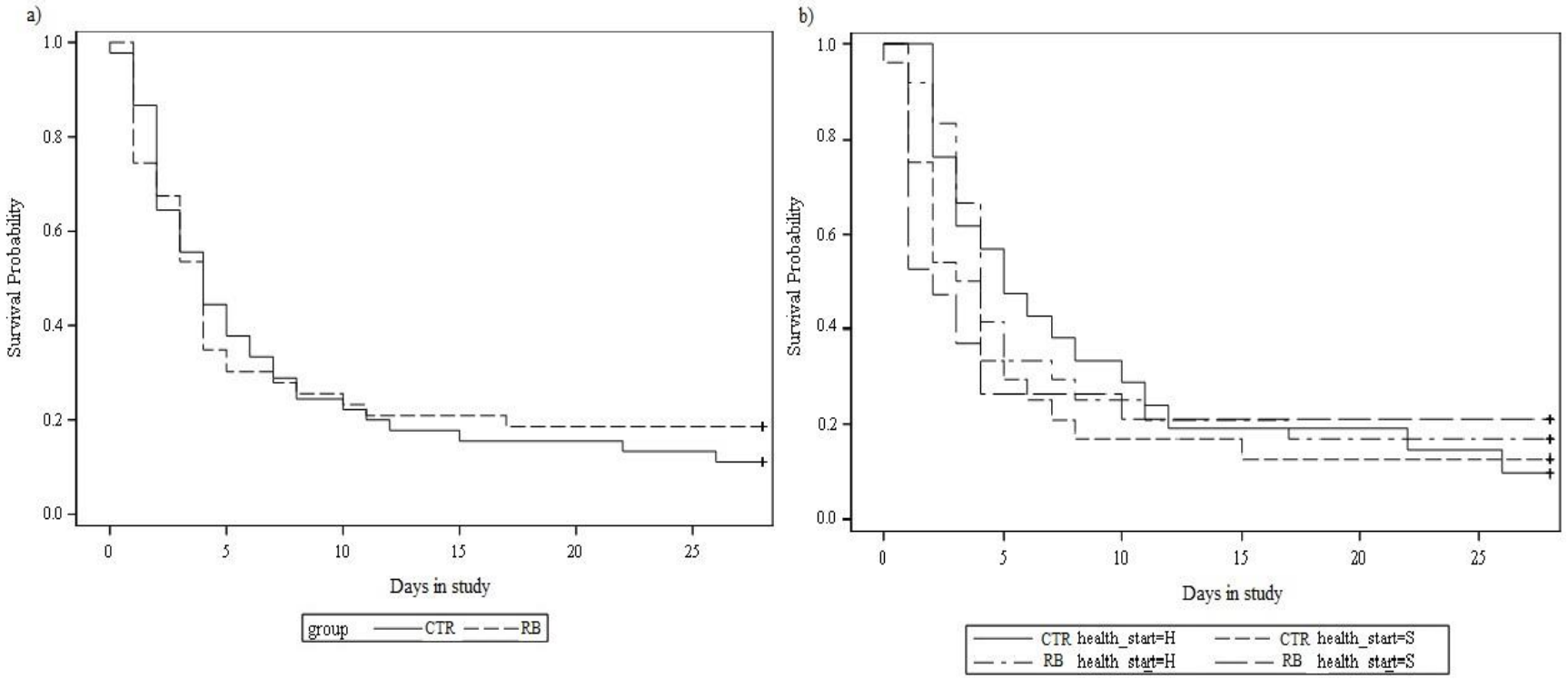

Figure 2.1: Comparison of time to first "moderate" disease status for control (solid line) and rice bran group (dashed line) (P=0.71); a). Comparison of time to first "moderate" disease status by health at enrollment and treatment groups. Healthy control (solid line), healthy rice bran group (Dashed and dotted line), sick control (dashed line), sick rice bran (line and dashed line), (P=0.08); b).

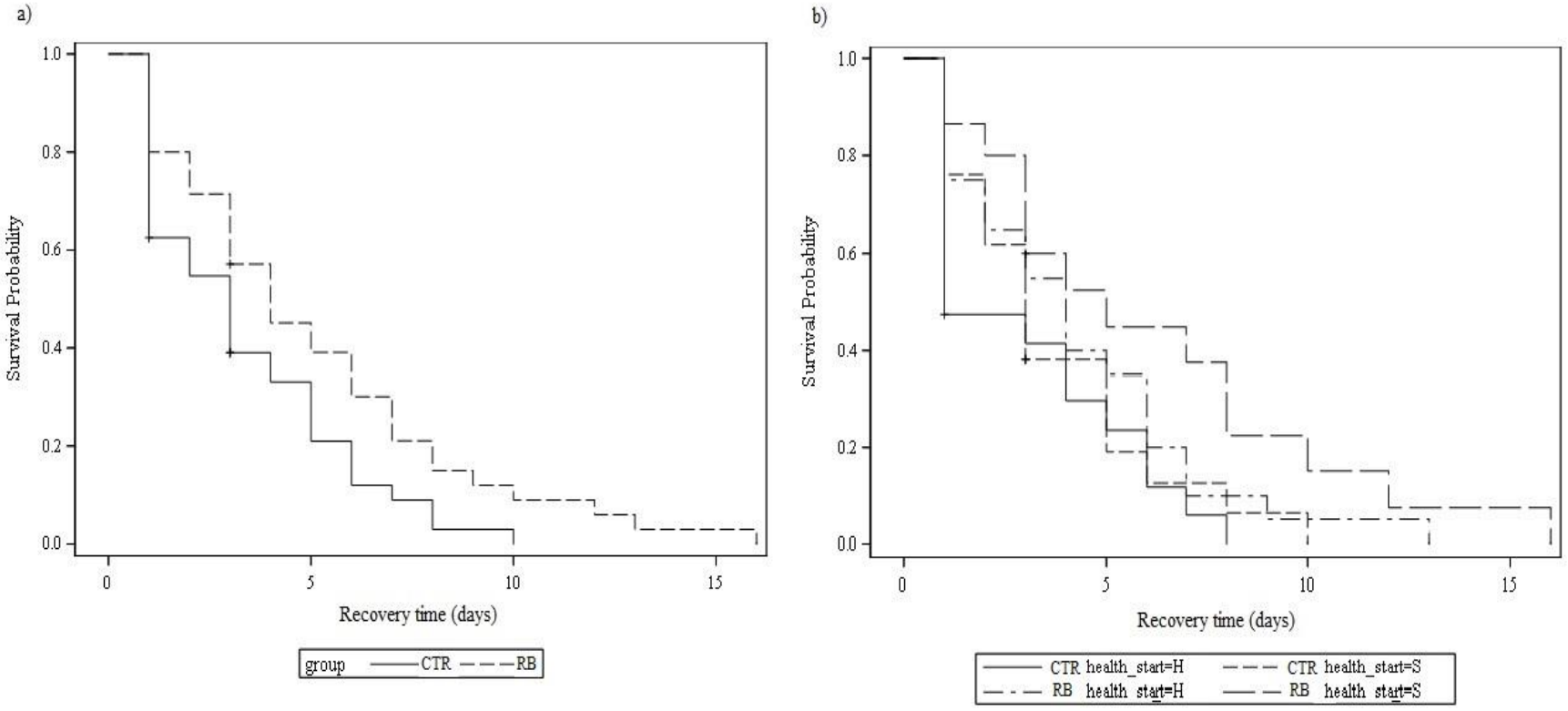

Figure 2.2: Comparison of time to recovery from a "moderate" disease status. control (solid line) and rice bran group (dashed line) (P=0.052); a). Comparison of time to recovery from a "moderate" disease status by health at enrollment and treatment groups. Healthy control (solid line), healthy rice bran (Dashed and dotted line), sick control (dashed line), sick rice bran (line and dashed line), (P=0.12); b).

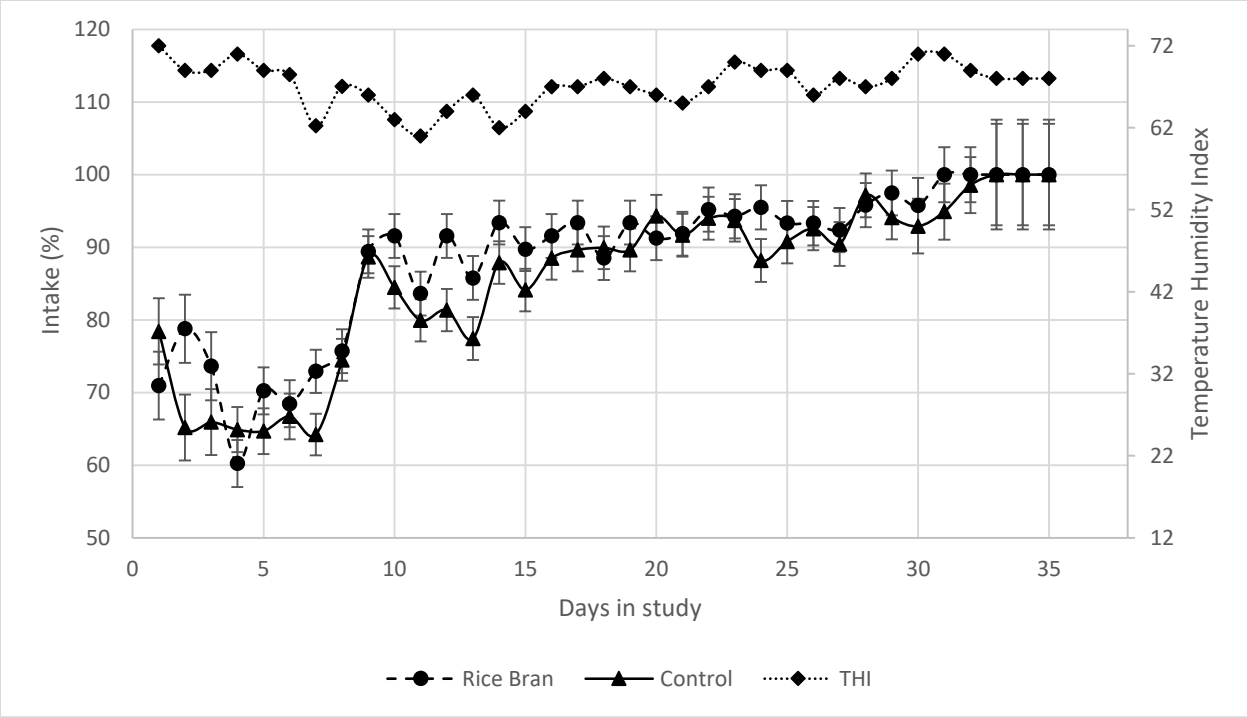

Figure 2.3: Average daily intake in control calves (solid line) compared to rice bran calves (dashed line); Daily temperature humidity index (THI; dotted line) is included (P=0.76)[1]

[1] P-value for the interaction between daily intake by treatment group and daily THI.

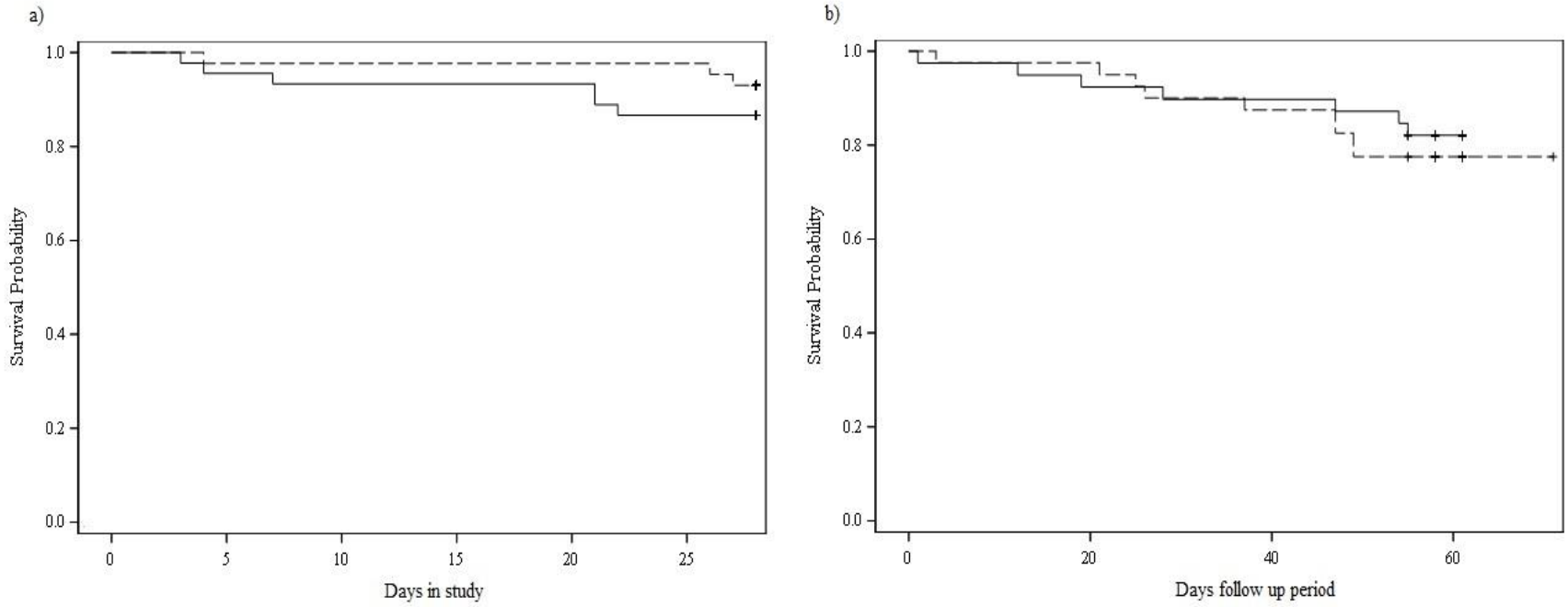

Figure 2.4: Comparison of time to death or culling for control group (solid line) and rice bran group (dashed line), during the study period (P= 0.29); a). From birth to weaning (P= 0.63); b).

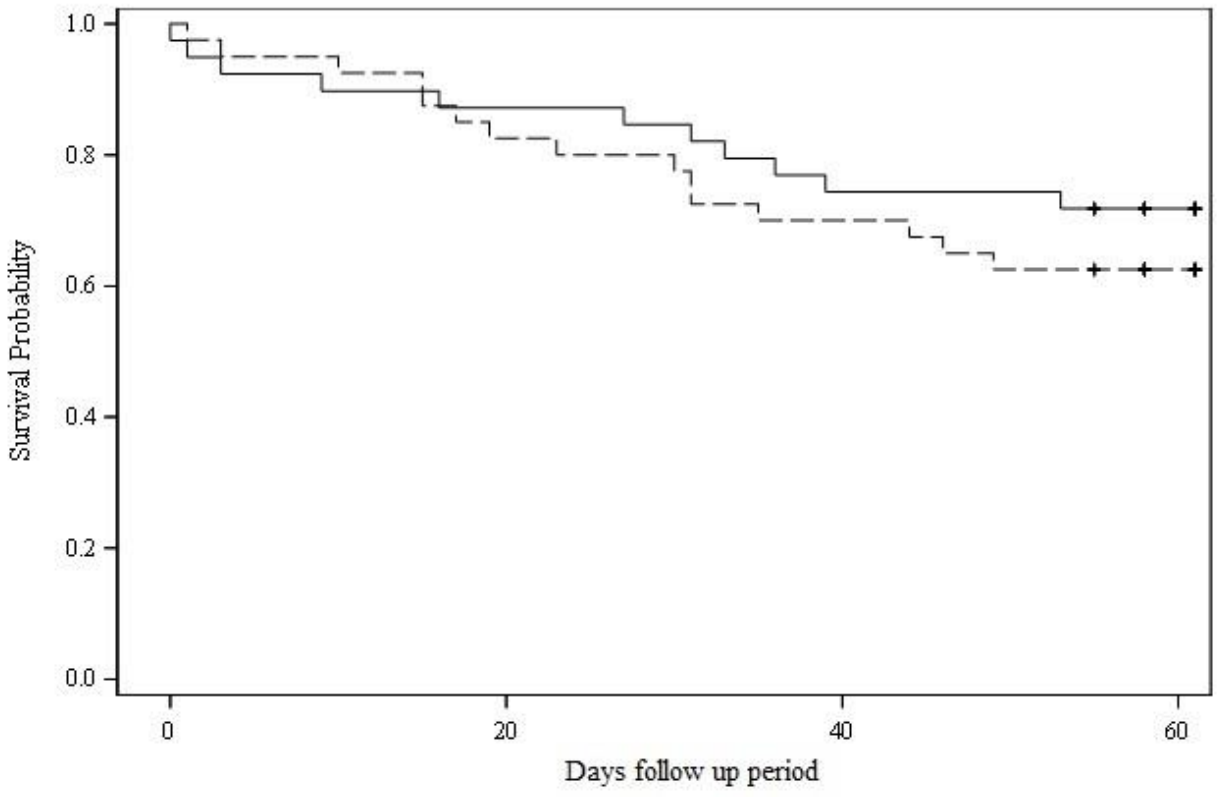

Figure 2.5: Comparison of time to first disease event during the follow up period, control group (solid line), rice bran group (dashed line) (P=0.43)

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CHAPTER 3: EFFECT OF AN AUTOMATED CALF BRUSH ON HEALTH, BEHAVIOR, AND PERFORMANCE OF RECENTLY WEANED HEIFER CALVES

Summary

Calf stress at weaning and after transferring to group pens is a concern in dairy farms. Favoring natural behaviors, such as grooming, may help to reduce this challenge. Our objective was to evaluate the effect of an automated brush on the performance, health, and behavior of recently weaned calves. A paired comparison design with 2 treatment groups (control [CON, n=81]; treatment [AB, n=81]) was performed. Organic Holstein heifer calves (94±7 d old) were monitored for 3 weeks after transferring from individual hutches to group pens. Four cohorts, considering one CON and one AB group (1 brush/pen, n=19-20 calves/pen) were enrolled sequentially. At enrolment, each calf was weighed and subject to a health evaluation. A 3-D accelerometer sensor was attached to the left ear. Continuous and excluding measurements (min/h) were generated for the following behaviors: “Not-active”, “active”, “highly active”, “eating”, and “ruminating”. Calves were weighted at the last day of the study. Behavioral data were summarized as daily averages of minutes per hour. In addition, total monitoring time was divided in 4 periods of 5 days and analyzed as hourly averages (min/h). Data were examined using repeated measures analyses ANOVA, considering day or hour as the time unit. No difference was found in average daily weight gain between treatment groups or in the incidence of disease. Significant differences were found for the interaction of time by treatment group, indicating greater values for CON calves on the time spent not-active (22.8±0.82 vs. 21.7±0.82 min/h; P=0.014) and lower values for the time eating (6.43±0.40 vs 7.01±0.40 min/h; P=0.01) during the 20 days in study. Hourly not-active time was greater for CON pens for Period 1 (18.3±0.78 vs 17.9±0.78 min/h; P<0.001) and Period 2 (24.7±1.08 vs 22.8±1.08 min/h; P=0.01). Similarly, differences in favor of the AB group were

found for time with high activity in Period 2 (10.3±0.57 vs 10.0±0.57 min/h; P<0.001), Period 3 (9.7±0.58 vs 9.1±0.58 min/h; P=0.049), and Period 4 (9.9±0.58 vs 9.6±0.61 min/h; P=0.02). Differences in eating time were found for Period 1 in favor of CON (8.3±0.51 vs 8.0±0.51 min/h; P<0.001) and Period 2 in favor of AB group (6.44±0.54 vs 5.48±0.54 min/h; P=0.003). Overall, 97% of the calves had a first interaction with the brush within 2.5 days with a mean (SE) of 7 (±9.6) hours after being transferred to collective pens. We conclude that the use of a mechanical brush after transferring calves to group housing had an effect on behavior, reducing inactive time, while increasing eating time.

Introduction

The weaning period is a stage in the life of dairy calves that is characterized not only by the change in diet, but also for a new social and physical environment that calves might confront and adapt to in a short period of time. The capacity of weaned calves to overcome these challenges and have a successful social interaction is bonded with the characteristics of their new housing system, for example space per animal, access to food and water, and shelter availability (Weary et al., 2008).

Self-grooming has been described as low resilience behavior that is compromised during disease. At the same time, grooming is an innate behavior that might help to cope with stress (Van Erp et al., 1994). For this reason and attending to the concerns of the dairy industry in regards to animal welfare, different grooming devices have been developed in recent years. Nowadays, a growing number of farms are providing automated grooming brushes to lactating cows (Georg and Totscheck, 2001), not just due to possible increments in production, but also as a good management decision to provide an enriched environment (Wilson et al., 2002). In addition, brushes specifically designed for young calves are gaining acceptance and might represent a good tool to help calves

to cope with the transitioning period during weaning. Enriched environments for young animals reared in confinement have positive effects in the time animals engage in locomotor play (Pempek et al., 2017). For example, in pigs it was reported that the effects of enriched environments in comparison with barren environments had positive effects in growth, feed intake, and in exploratory behavior, reducing inactive and aggressive times (Beattie et al., 2000).

Changes in behavior, health status and performance are reliable parameters to assess the effectiveness of different management decisions or environment improvements.

However, behavior in calves has not been fully explored and published data is limited (Swanson and Harris, 1958; White et al., 2008; Burfeind et al., 2011; Pempek et al., 2017). The observation of animals for behavior evaluation is time consuming and it might be subjective, as it usually relies on recording systems or visual inspection that just cover parts of the day (Toaff-Rosenstein et al., 2017).

The dairy industry is highly technified and the development of devices helping to predict diseases or physiological changes by measuring core behaviors (eating, rumination and resting time) is a reality for adult cattle. The use of accelerometer sensors with continuous recording it is an accurate method to obtain objective information for long periods of time. In dairy calves, the use of these devices is not common at the farm level. However, these new technologies open a new approach to do research (White et al., 2008; Trénel et al., 2009; Hill et al., 2017; Hodson and Timms, 2017).

Therefore, we hypothesized that the addition of a grooming device in collective pens of weaned calves will improve performance, reduce the presentation of diseases and it will have a positive effect in core behaviors of the animals. The specific objectives of this study were to evaluate the effects of an automated grooming brush on behavior, performance, and health of

recently weaned calves transferred from individual to collective housing, through the use of 3D accelerometer sensors. A secondary objective was to describe the dynamics of the interaction of calves with the automated brush through video recording.

Materials and Methods

This study was conducted in a commercial organic certified calf yard, located in Northern Colorado. Weaned calves were managed during the study period according to the guidelines set by the Institutional Animal Care and Use Committee of Colorado State University (Protocol ID: 17-7236A).

Animals, Housing and Feeding

A total of 162 recently weaned organic Holstein heifer calves were enrolled for this study. Data was collected from October 29th, 2017 to December 29th, 2017. At enrollment, calves were 94 ± 7 days of life. Weaned heifers were followed for a period of 3 weeks to evaluate the effects of an automated grooming brush available in their collective pen on performance, health and behavior.

Management and housing before weaning were the same as described in the chapter "Evaluating the effects of Stabilized Rice Bran in pre-weaned dairy calves". Briefly, calves were transferred to the rearing facility during the first day of life. Heifer calves were housed in individual polyethylene hutches (Agri-Plastics, Stoney Creek, ON, Canada) with straw bedding and a wire gate enclosure. Calves had visual but no physical contact with other animals until weaning.

Pasteurized milk was provided 3 times a day (6 AM, 1 PM and 7 PM) in 2.8 L bottles (E-Z Nurse^{TM}). The weaning process was progressive and started at 73 ± 7 days of age and was completed in a 3 weeks period. At the beginning of the first week of weaning, the night feeding was suspended. At the beginning of the second week of weaning, the noon feeding was suspended.

Lastly, at the beginning of the third week of weaning, the morning feeding was suspended. During this week, calves stayed in their respective individual hutches to be monitored before being transferred to collective pens.

The decision to move healthy weaned calves, from hutches to collective pens, was made by farm trained personnel, based on space availability, consumption of calf starter (1.8 to 2.2 kg per day), weight (minimum 72 kg) and age. Animals were housed in groups of 20 to 22 calves.

A clean livestock trailer, with capacity for 40 calves, divided in 4 internal compartments, was used to transfer healthy weaned calves to group housing. Two trained workers had the responsibility to load each calf into the trailer following the farm protocols. A third person drove the trailer.

Once the trailer had 40 animals, calves were moved to the collective pen area, located in the same rearing facility. Calves were slowly and carefully unloaded into the collective pens, with the use of flags. Once all calves were in their respective pens, the personnel continued loading animals depending on space availability and management instructions.

The collective pen building area was 128 m x 24 m. Nine pens conformed the total area. A solid back wall and a ceiling covering the bedding area functioned as shelter from environmental conditions. Clean and dry straw bedding was provided for each pen and it was replaced as needed.

Eight pens (24 m x 16 m wide) were constantly used. The covered area measured 7 m x 16 m and the feeding area per pen was 16 m x 3 m. The 9^{th} pen was used to maintain and monitor weak calves and was half size of the size of the other pens.

Feed was provided in a feed bunk lane. The daily ration consisted in an increasing amount of 3.6 to 4.5 kg of calf mix (75% calf starter, 25% TMR; A2.1, A3.1) provided each day at 7 AM and 5 PM. Two water troughs were available per pen and they were cleaned regularly following

the farm SOP. Calves were housed in collective pens for 3 weeks. Four out of the 9 pens had one automated grooming brush (treatment pens) distributed every other pen.

The vaccination program after weaning included the administration of Ultravac 8 (*Clostridium chauvoei, Cl. Septicum, Cl. Haemolyticum, Cl. Novyi, CI. Sordellii, Cl. perfringens* Types C and D. Zoetis Inc. Florham Park, New Jersey, USA) and Bovishield Gold FP5, L5, HB (IBR, BVD Types 1 and 2, PI_3 and BRSV, *Leptospira borgpetersenii* serovar hardjo-bovis, *Campylobacter fetus, L. Pomona, L. grippotyphosa, L. canicola,* and *L. icterohaemorrhagiae.* Zoetis Inc.*)* at 120 days of life.

Once a day, weaned calves were monitored by trained personnel to detect sick animals. All sick animals were moved to large hutches conditioned in an isolation area and housed in groups of 3 or 4. Animal husbandry and treatments provided were performed as specified in the farm protocols. After the three weeks period was completed, calves were moved to another rearing facility.

Experimental design and treatments groups

A paired comparison study with two treatment groups (TG) was designed. Four cohorts of calves were monitored for this study. One control (CON) and one treatment group (automated brush= AB) were enrolled simultaneously per cohort, depending on weaning dates.

The first group was enrolled in October 29th 2017, and consisted in 44 calves (CON=22, AB=22). The second group was enrolled in November 8th 2017 (CON= 19, AB=20). The third group was enrolled in November 17th 2017 (CON=20, AB=20). The fourth group was enrolled on November 27th 2017 (CON=20, AB=19).

The enrollment was performed at the same day that calves were fully weaned, although behavioral and health records were analyzed since the day of transfer to collective pens. Average age was 94 ± 7 days and weaned dairy heifers remained housed in the individual hutches for 10 ± 3 days after weaning.

All calves were subject to a clinical evaluation at enrollment and only healthy animals were included. Each enrolled calf was weighted using a mobile crate scale (LFT-700S, W-W Paul Scale, Duncan, OK). Additionally, the individual weight of each calf was obtained at the end of the study period, before the animals were transferred to another facility.

Accelerometer sensors

Calves were safely placed in the crate scale and weighted. The left ear was examined for irritation or infection and the farm ID ear tag was removed from calves with not irritated or infected ears. After a disinfection with 7% iodine solution, a 3-D accelerometer sensor (Cow Manager SensOor, Agis, Harmelen, The Netherlands) was attached replacing the farm ID. All enrolled calves were tagged.

Following the manufacturer recommendations the 3-D accelerometer sensor was placed in the center of the left ear, which coincided with the perforation of the farm ID. Therefore, with a few exceptions, there was no need for re-perforating the ear. The sensor was attached with a male-female button. To allow for a better identification of the calves in the collective pens, the ID number of each calf was written with a permanent marker in the back of the right farm ID tag.

Each 3-D accelerometer sensor had an individual number that was linked to a specific calf in the system's software. The system, based in proprietary algorithms (not published data), provided exclusive measures in minutes per hour for "active", "high active", "not-active", "eating"

and “rumination” time. Additionally, health alarms were generated. For the purpose of this study, only “suspicious” and “very sick” alarms were considered.

A solar power antenna was installed in the collective pen area to maintain the system operating correctly. A master laptop was located in the rearing facility office. According to recommendations of the manufacturer, and for an optimal performance of the system, calves were tagged 7 days before the expected transfer date to collective pens. This date coincided with the last day of the weaning process and this was the date considered as enrollment, although the day of transfer to collective housing was considered day 1 for the analysis of behavioral data. During this period, alarms and information related to each calf was checked daily to resolve any possible problem with the system, before transferring the animals to group housing.

The removed farm ID tags were stored in sealed bags and located in a clean area under management supervision. When the study period was completed, and using the scale crate as calf chute, the 3-D accelerometer sensors were removed and the ears were examined for any abnormality. After disinfection with 7% iodine solution, the farm ID was re-attached in the same perforation.

All heifer calves that left the collective pens due to disease had the 3-D accelerometer sensor removed. The animals were delinked from the system and considered out of the study.

Automated grooming brush

An automated grooming brush was used in each of the 4 AB pens (Comfort Brush^{TM} for calves, Future Cow^{R}, Longwood, FL). The brush dimensions were 60.9 cm long and 45 cm diameter, with 360° of horizontal movement and 45° of vertical swing. The brush ramps up slowly to not scare the calf and a motion sensor, automatically activates the rotation of the brush. Each brush was located under the ceiling of the building and in the middle of the pen. Each brush was

attached to a metallic column, part of the supportive structure of the pen. Brushes were installed 60 cm above the ground. The motion sensor was facing the bedding area.

Brushes were located in every other pen, therefore CON and AB group were located contiguously. To avoid mixing of groups, farm personnel was instructed to keep all communication gates closed with chains. In total 4 out of 9 pens, that conformed the collective housing area, were used sequentially to house the 4 CON and 4 AB groups of calves enrolled for this study.

Recording camera system

To record the interaction of the AB calves with the automated grooming brush by EG, 2 digital video recording (DVR) units with capacity of 1 Terabit and 4 channels were used. Each DVR was used to record 2 AB pens. Cameras were weatherproof with night vision up to 30 meters (1080p HD surveillance, model: dvr4-4575, Swamm). Two cameras were installed per AB pen, and they were programmed for 24 hours recording.

One camera was installed focusing on the brush area in an angle of 45° and 50 cm above the brush in diagonal line. This camera allowed the visualization of each calf ID and the physical contact with the brush. The camera was strategically positioned to permit the recording of the complete automated brush. The second camera was installed 8 meters from the brush and allowed the visualization of the pen with exception of the feeding area. It was used as support of the fist camera, providing a wider view of the brush area.

Cameras were attached to a piece of wood and held with 2 metallic adjustable rings to sturdy and secure structures. DVR, cables and connections, were placed in an intermediate point between two AB pens and protected of weather condition with a plastic storage box. The storage box was placed 4 meters above the ground. All cables were placed in the ceiling of the barn to do not disturb normal duties.

Collective pens

The day of transferring to the collective pens, a group of 4 people, according to farm SOP and following the TG assignment list, placed the animals in a livestock trailer with capacity for 40 weaned calves. The trailer had 4 mobile internal gates that allowed place 10 animals per compartment.

Control calves were loaded first from hutches to the trailer. Subsequently, automated brush calves were moved to the trailer. Every 10 animals the compartments were closed to prevent falls and mixing of groups.

Collective pens were located in the same rearing facility and the animal transport, once the last calf was loaded, lasted less than 15 minutes. Farm personnel unloaded the calves from the trailer using flags. Weaned calves walked through a small lane way into their respective pens. All handling was performed by trained personnel, and lasted, approximately 1 hour.

Group 1, 2, 3 and 4 were moved 7, 9, 10 and 14 days after enrollment, respectively. Enrolled calves were monitored in collective pens for 18 to 20 days depending on the group. A daily health check was performed by trained personnel. As it was mentioned before, vaccination was performed before moving the calves to other rearing facility in the same complex.

CON and AB calves experienced similar handling and housing conditions except for the presence of the automated grooming brush. Detection of clinical disease and treatments administered to calves by farm personnel were obtained from farm records.

Statistical Analysis

Data were analyzed using SAS statistical software (9.4, SAS Institute Inc., Cary, NC), with calf as experimental unit of analysis. Models included treatment group (TG= CON, AB) and

enrollment group by date (EG= 1 to 4) unless otherwise specified. Least square means (LSM) were calculated for weaning/initial weight, final weight, weight gain and ADG using PROC GLM.

Differences in frequencies of animals that left the collective housing due to disease were tested using PROC FREQ and logistic regression (PROC LOGISTIC).

Kaplan Meier survival analysis (PROC LIFETEST) was performed to determinate group differences in the time to the occurrence of the first disease and treatment. Wilcoxon test was used to determine statistical differences. In addition, differences in the number of calves that were detected sick for a first time by farm personnel was analyzed using logistic regression (PROC LOGISTIC).

Kaplan Meier survival analysis (PROC LIFETEST) was performed to determinate differences by group in the time to the first alarm provided by the 3-dimmentional accelerometer sensor ("suspicious" and "very sick") and in the time to the first "very sick" alarm. Wilcoxon test was used to determine statistical differences. In addition, differences in the number of calves that presented health alarms were analyzed using logistic regression (PROC LOGISTIC).

In addition, a Poisson regression (PROC GENMOD) was performed to analyze the number of health alarms per calf and the differences between TG. Alarms spaced for at least 3 days were considered in the analysis.

To analyze the time that weaned calves spent as "not-active", "active", "high active", "eating" and "ruminating" during the 20 days in study, the data generated by the sensors was averaged by day in the study (1 to 20) for each calf. Least square means were estimated for each response variable using repeated measures ANOVA (PROC MIXED). The model included TG, day number, and the interaction between TG and day number.

To analyze calf activity by hour of the day (0:00 to 23:00h) and TG, the monitoring time was divided in 4 periods (Period 1: day 1 to day 5; Period 2: day 6 to day 10; Period 3: day 11 to day 15, and Period 4: day 15 to day 20). Repeated measures ANOVA (PROC MIXED) was performed, to estimate least square means. The model included TG, time and the interaction between TG and time.

PROC FREQ was used to calculate mean, median, minimum and maximum time to the first interaction with the brush by EG. In addition, the time that each calf spent in the brush in the first interaction was measured (< 1 minute or ≥ 1 minute) and frequency was calculated. A description of the order of body contact area with the brush was performed. Statistical Significance was stablished at $P \leq 0.05$ and tendency at $0.05 < P \leq 0.1$.

Results

A total of 162 recently weaned Holstein heifers were enrolled in 4 sequential enrollment groups. Calf ADG did not differ between TG (P=0.69, Table 3.1). Additionally, no differences were observed in ADG by EG (P= 0.21).

In total, 25 animals left the study due to disease (CON=16, AB= 9, Table 3.2) during the 20 days of collective housing. However, no difference was found (P=0.12) for the odds of leaving the study due to disease (OR=2.01, 95% CI= 0.82 - 4.9) for CON calves in comparison with AB calves or by EG (Table 3.3). During the study period, 29 calves were treated for respiratory disease (CON= 19, AB= 10, 18% of the enrolled calves; Table 3.4). Control calves from EG1 presented the greater number of sick animals. On the other hand, AB calves from EG4 did not present any disease event. A tendency for significance was determined (P=0.064) for greater odds of sicknesses detected by farm personnel (OR= 2.17, 95% CI= 0.95 - 5.18) for CON calves in comparison with

AB calves. No differences were found for the time to the first disease detection and treatment by farm personnel for TG (P=0.12; Figure 3.1).

Overall, 117 animals reported at least one health alarm generated through the 3D accelerometer sensors. These calves represented 72% of the animals enrolled. The alarms included "suspicious" (23 animals) and "very sick" status (94 animals). Enrollment group 1 reported the lower number of alarms in comparison with the other groups (Table 3.5). However, no differences (P= 0.36) were found in the odds of presenting a health alarm by TG and EG (OR= 1.38; 95% CI= 0.68- 2.80). In addition, the survival analysis did not reveal differences in the time to a first health alarm between TG (P=0.77; Figure 3.2a). At day 10 in study, 70% of the CON and AB groups had generated the first health alarm. Furthermore, 58% of the animals enrolled presented at least one "very sick" alarm during the time they were housed in collective pens. EG1 generated the minimum number of alarms in comparison with the other groups (Table 3.6). However, no differences (P= 0.49) were found in the odds of presenting a "very sick" alarm by TG and EG (OR= 1.24; 95% CI= 0.65- 2.36). The survival analysis indicated not significance for the time to a first "very sick" alarm by TG (P=0.48; Figure 3.2b) and 60% of the "very sick" alarms were already reported at day 14. Regarding the number of alarms reported by calf, the Poisson regression did not reported differences in the number of alarms per calf and per TG (P=0.44, Table 3.7).

When the behavioral data was averaged by day, calf ID and behavior variable, the repeated measures analysis indicated a significant effect for the interaction day x TG for the variables "not-active" and "eating" time. Control calves spent more time "not-active" in comparison with AB calves (P=0.014; 22.8±0.82 vs. 21.7±0.82 min/h; Figure 3.3; A3.2). Additionally, AB calves spent more time "eating" (P= 0.012; 6.43±0.40 vs 7.01±0.40 min/h; Figure 3.4; A3.3) during the time period of 20 days. No differences were found for the interaction between day number and TG for

"active" time (P = 0.36; 5.7±0.31 vs 6.2±0.31 min/h; A3.4a), "high active" time (P = 0.12; 10.50±0.37 vs 10.26±0.38 min/h; A3.4b), and "ruminating" time (P = 0.28; 15.24±0.51 vs 14.38±0.51 min/h; A3.4c).

When the behavior variables were analyzed by hour within 5 days intervals, the repeated measures analysis indicated a significant interaction effect between hour of the day and TG for "high activity" in Period 1 in favor of CON calves (P<0.0001; 13.51±0.48 vs. 13.81±0.48 min/h Figure 3.5a). Period 2 (P<0.0001; 10.3±0.57 vs 10.0±0.57 min/h; Figure 3.5b), Period 3 (P=0.049; 9.7±0.58 vs 9.1±0.58 min/h; Figure 3.5c), and Period 4 were in favor of AB group (P=0.02; 9.9±0.58 vs 9.6±0.61 min/h< Figure 3.5d). Additionally, the interaction effect was significant for "not-active" time in Period 1 (P=0.0002; 18.3±0.78 vs 17.9±0.78 min/h; Figure 3.6a) and Period 2 (P=0.01; 24.7±1.08 vs 22.8±1.08 min/h; Figure 3.6b), suggesting that CON calves spent more time not-active than AB calves during the first 10 days after being transferred to the collective housing. The interaction effect between hour of the day and TG differed for the response variable "eating" time for Period 1 (P=0.0002; 8.3±0.51 vs 8.0±0.51 min/h; Figure 3.7a) and Period 2(P=0.003; 6.44±0.54 vs 5.48±0.54 min/h; Figure 3.7b), indicating that AB calves spent more minutes per hour eating.

Furthermore, analyzing the data by hourly intervals indicated how the behavior of the calves was changing through the day. The "active" time peaked between 9:00 and 14:00 h for the 4 periods (A3.5). "High active" time peaked between 7:00 and 10:00 h, also another peak was observed at 16:00 and 17:00 h for the 4 periods (Figure 3.5). "Eating" time peaked between 7:00 and 8:00 h (A3.6) and "rumination" between 4:00 and 5:00 h, steadily increasing from 1:00 AM for all periods analyzed (A3.7). The peak in "not-active" time was observed in the interval 2:00 and 4:00 h (A3.8).

For the video recording data, 3 groups of animals were observed (n=65) for 5 days. Due to technical problems, a different cohort of animals was followed between January and February 2018. With the exception of 2 calves, all animals used the automated brush daily (97%).

The first interaction with the brush considered any physical contact when the calf was aware of the brush. Calves that touched the mechanical brush when performing other activity (playing or running) were not considered. The first area of contact was the nose and the head for all calves. After the first contact, calves started to use the automated brush on the neck, the thorax and the abdomen. The areas less covered during the first days of use were the posterior legs and the rear area. The utilization of the grooming brush was constant during day and night.

Once the transfer to collective housing was finished, the first calf that used the brushed took between 5 and 10 minutes depending on the group. All calves, with exception of 2 animals in group 2 used the brush in the first 2.5 days after being transferred (Table 3.8). Overall, 57% of the calves spent ≥ 1 minute in the first contact with the brush (Table 3.9). In final inspection of the left ear, 4 calves presented irritation in the perforation point and one heifer a dropped ear.

Discussion

Our study centered in the effects of an automated grooming device on recently weaned calves housed in groups for the first time. The implementation of grooming devices in the dairy industry has occurred in parallel to the study of grooming habits in adult cows in different productive stages (Georg et al., 2001; DeVries et al., 2007; Newby et al., 2013). The use of automated brushes has been linked with low resilient behaviors and the prediction of diseases or stress and discomfort in adult dairy cows (Mandel et al., 2013; Mandel et al., 2017; Mandel et al., 2018 ;). Even though dairy calves experience stress in different stages of their life, to our knowledge, the investigations made in the effects of enrichment environment it is not extensive,

and the repercussion of grooming devices in health, ADG, and behavior has not been fully covered. Limited published data exists describing the use and behavior of the calves towards brushes (Pempek et al., 2017; Toaff-Rosenstein et al., 2017).

Our results indicated no effect of the brush on ADG. Consistent with this, other enrichment strategies, such as pair housing of pre-weaned calves in furnished hutches had not influence in body weight and ADG at weaning (Pempek et al., 2017). However, in our study, an important consideration has to be done regarding the time calves were housed with the brush. Although this is a period of stress due to weaning, grouping and new housing, calves spent in total between 18 to 20 days in these pens and this time might be insufficient to assess differences in ADG, especially if a transitional period is included in the total exposure period.

The weaning process and the change to collective housing is a stressful period that can lead to respiratory disease (Gorden and Plumer, 2010; Roth et al., 2009; McGuirk, 2008; Callan and Garry, 2002). It has been reported that respiratory problems are the most common disorder in weaned calves, with a morbidity of 11.2% (USDA, 2011) accounting for the 46.5% of death losses (USDA, 2007).In our study, 18 % of the enrolled calves exhibited clinical respiratory disease, as detected by farm personnel. The number of calves from the AB group treated for respiratory problems was smaller compared to CON calves, even though no significant differences were found. The majority of sick calves were identified and treated between day 5 and 13 after transferring to collective housing.

The manufacturer's algorithm (not published data) for health alarms was created for adult cattle and has not been validated for calves. Consequently, when we compared data provided by farm records, we detected a difference in the number of animals detected and treated for clinical disease and animals that were "suspicious" or "very sick" according to the alarm system.

Furthermore, personnel in charge of checking weaned calves health was highly trained and from the several visits made for our group during the study period, the inaccuracy of health alarms was evident. Hodson and Timms (2017) reported the same problem with alarms in pre-weaned dairy calves. As the behavior of calves is different from that of adult cows, health alarms were constantly generated. In our study, not all the clinically sick calves were detected by the system. Moreover, the first days after transferring calves to collective housing, an important number of "heat" alarms were received, which was associated to an evident increase in "high activity". Therefore, for a reliable information the algorithms might be modified in order to represent the behavior of calves.

Relevant to this research, the 3D accelerometer sensors used in this study were validated for the use with dairy calves for rumination, eating, and activity behaviors. Health alarms and temperature recordings were not assessed for the authors (Hill et al., 2017). Additionally, Hodson and Timms (2017), using the same sensors, described that the raw data was representative of animal movement, behavior, and changes detected in health. Contrary to the findings made by Hill et al. (2017), our calves did not present problems with dropped ears and the irritation in the attachment area was minimal. The reasons might be linked to the age of our calves (older) and to the removal of the farm ID.

In adult dairy cows, accelerometer sensors have been used as a tool to determine changes in the behavior of animals to support health and to predict diseases, heat or parturition (Rutten et al., 2013). To our knowledge, published data for the use of accelerometers in calves, as a tool to measure behavior patterns and the implications on health is scarce and most of the studies performed related with behavior in calves are through the use of video recording or direct observation and usually they do not include 24 hours observation (Pempek et al,. 2017; Zobel et al., 2017; Bak Jensen el al., 1998; Wilson et al., 2002).

When the raw data generated by the sensors was analyzed by day, our results suggested that AB calves spent more minutes per hour "eating" and less minutes per hour "not-active" ("not-active" time was a measurement that excluded all the other possible measurement taken by the sensors). This difference was more evident from day 6 to 20 in study, which might be a consequence of an adaptation period in this transitioning time. At commingling, calves were still experiencing the weaning distress and the group housing may lead to competition for resources and hierarchy (Hulbert and Moisa, 2016; Jensen and Kyhn, 2000; Krachun et al., 2010), even though the social interaction and space allowance may help them to overcome this transitioning period.

Our results indicated that, after the first week of group housing, CON and AB calves stabilized the amount of time spent in each activity. The main difference was seen in the time calves spent "high active" and "not-active". The "high active" time decreased continuously from 15 min/h in the first 7 days to 9-10 min/h in most of the days after the first week in the collective housing. Conversely, "not-active" time was increasing from 17 min/h to 21-22 min/h depending on TG. Also "eating time" fluctuated in the first 7 days after transferring the calves. First day "eating" time was similar for both TG (7 min/h). From the second until the fourth day was the period with the highest amount of time spent eating (9 to 9.5 min/h) to later decrease until a stable time of 6.2 min/h for CON calves and 7 min/h for AB calves. On the other hand, "rumination" and "active" time stayed in the same range during all the study period.

Furthermore, as previously discussed, most of sick animals were detected by farm personnel between day 5 and 13 in the collective housing. From our results, the first week after transferring calves from individual to collective housing is a transitioning period that might to require special attention on rearing facilities to detect disease and re-organize calves groups when

needed. As mentioned by Moran (2002), weaned heifers required less attention and efforts than pre-weaned calves and lactating cows and for this reason they tend to be relegated. Knowing the importance of the transitioning period after weaning, farm protocols and research is encouraged.

There is very limited published data in regards to calf behavior and the use of automated brushes in calves and no published data is available where both topics are evaluated simultaneously. However, White et al. (2008) described the changes in behavior of bull calves after castration through the use of 2D accelerometer sensors and continuous monitoring. The conclusions were that castrated calves spent 86% more time standing than control calves, but no differences were found in eating time. Borderas et al. (2008), exposed calves of 3 and 20 weeks to a low dose of bacterial endotoxin. Calves that were administered the bacterial endotoxin, had a reduced rumination, eating, self-grooming time and an increased time lying inactive in comparison with control calves.

As stressors might have an effect in behavior, the provision of enrichment devices might have repercussions in behavior and health of calves, as well. As suggested by Georg and Totscheck (2001), brushes reduce frustration or stress due to boredom when animals are housed in intensive productive systems.

On the other hand, there are published studies were eating and rumination time in pre-weaned and weaned dairy calves were described. Our results, indicated that CON calves spent 14±0.52 min/h “ruminating” and 6.43±0.40 min/h. “eating” and AB calves spent 15±0.52 min/h “ruminating” and 7.01±0.40 min/h “eating”. Burfeind et al. (2011) measured rumination time by observation in 3 period of 2 hours, finding an average of 26± 14 min/2 h for calves 95± 10 days old and 30±18 min/2 h for calves 185±1 day old.

However, the period when the information was collected was not specified and calves were housed individually. Our TG spent more time ruminating than 95 days old calves and AB calves spent similar ruminating time than calves 185 days old.

In an observational study, rumination, eating and lying behavior were assessed by visual inspection during a full day in dairy calves of 9 to 107 days old (Swanson and Harris, 1958). One of the main differences with our design was that calves were housed individually with no physical or visual contact with other calves. Holstein heifers 93 days old spent as an average 6 h ± 16 min/day eating and drinking, 4 h± 11 min /day ruminating, and 14 h±23min/day lying. Holstein heifers 107 days old spent 6.5 h± 10 min/day ruminating, 3.8 h±15 min/day eating and drinking, and 14.5 h±22 min/ day lying down. Our calves being slightly older, as an average spend around the same amount of time ruminating, eating time was greater for Swanson and Harris (1958) calves but they included drinking time. In relation with resting time, there is not a description of the parameter, therefore, it is not proper compare with the inactive time obtained for our calves.

Even though we did not find differences between the interaction TG x time (hour of the day), for all the variables analyzed, the information obtained showed an interesting pattern in the behavioral routines of recently weaned calves by hour. To our understanding, there is not published data regarding this topic. Trénel et al. (2009), described lying, standing and movement behavior in calves with the use of Ice Tag^{R} sensors (IceRobotics LTD, Edinburgh, UK.). The study was a validation of the sensors for the use in calves. Their findings suggested an overestimated activity in comparison with recording videos. Also, there is not description of the behavior for the 12 hours of video recording. As it was mentioned before, there are studies that assessed eating time, rumination and laying time in certain amount of hours during the day, or an average time per day, without the description of patterns during the day (Burfeind et al., 2011; Borderas et al., 2008;

Swanson and Harris, 1958). Furthermore, White et al. (2008), used a 2D accelerometer to measure changes in behavior after castration in beef calves. However, the information was organized as percentages of changes in activity before and after castration.

The dynamics of the readings provided by the 3D accelerometer sensors were in agreement with the practices stablished at the farm. For example, the peak eating time happened at the time when farm personnel fed fresh TMR to all collective pens between 7:00 and 8:00 h. At this time, during the first days after transferring the animals, all calves were moved towards the feed bunk and gates were closed for about 20 minutes to encourage eating and to teach the calves where the feed was placed in their new housing. Also, the second peak of eating happened around 17:00 h which was the time when the feed bunk was re-filled with TMR. The eating patterns were consistent through the 4 periods of 5 days. The only difference was the time spent by calves eating. In period 4, both TG spent the highest amount of minutes per hour eating between 7:00 and 8:00 h. It is worth mentioning that calves exposed to automated brushes spent more time eating throughout the day. As expected, the hourly pattern of rumination was opposite to that of eating. The lowest period of rumination coincided with the peak in eating, between 6:00 to 9:00 h. Also, a decreased amount of minutes spent in this activity occurred between 15:00 and 17:00 h. During the evening and early morning hours a progressive increase in rumination was observed, with a peak between 16:00 and 17:00 h. Nonetheless, Calves from the two TG spent similar time ruminating in the 24 hours period.

As the measurements for “not-active”, “active” and “high active” generated by the sensors were excluding from each other, this 3 variables had peaks and patterns that were complementary. For example, the observed “not-active” time had the higher value during 2:00 to 4:00 h. Conversely, it remained in its lowest values between 7:00 and 9:00 h (eating time). Calves were

more “active” from 10:00 to 14:00 h for all the periods analyzed and activity remained steady between 14:00 to 0:00 h. The lowest records for “active” time were between 1:00 and 4:00 h. In addition “high activity” peaked between 7:00 and 10:00 h.

High activity it is a measure used in adult cows to detect heat. Therefore, it may be a conjunction of different behaviors (as a decreased rumination or smaller time spent inactive). As the algorithms are unknown to us, it is not possible to interpret this parameter. Even if patterns were similar along the day, calves in the AB pens spent more time in high activity and less time not-active than control calves, indicating that the housing with a grooming device encourage core and low resilient behaviors.

From the video recording, 97% of the calves used the brush in a lapse of 5 days. Toaff-Rosenstein (2017), working with 7 to 9 month old beef calves, reported that all animals made at least one physical contact with the brush in the 5 days they were housed with the brush. A difference with our design is the number of animals enrolled and also the number of animals housed per pen (n=16; 8 calves/pen). Georg et al. (2007), found that 98% of 72 dairy calves, 40 to 98 days old, used the brush in the 3 different periods analyzed.

On the other hand, it has been described that 56.9% and 79% of adult cows used the brush within the first 24 hours (DeVries et al., 2007; Georg and Totscheck, 2001) and 93% of cows use the brush within the first 7 days after the installation with an average time of 45.5 ± 64.1 hours (DeVries et al., 2007). From our results our mean times per group were consistently smaller than those described for adult cows. DeVries et al. (2007) described that cows increased the grooming time in 508% when the mechanical brush was added to the pens of lactating cows and the frequency of grooming increased in 226%. Pempek (2017), described when a stationary brush was

added to a furnished hutch, calves reduced the time scratching themselves against fixtures. Also, the brush was preferred over other items, such as a rubber chain, a calf lollie and 2 artificial teats.

Head and neck were the first areas of contact with the automated brush. In addition, head and neck were the areas of higher contact during the first days in the collective housing. Depending on the calf and the time spent on the automated brush, heifers moved the brush to other areas as thorax and abdomen or the rear area. These findings are in agreement with previous studies (DeVries et al., 2007; Georg and Totscheck, 2001; Toaff-Rosestein et al., 2017; Zobel et al., 2017). As it is presented in our results, more than the half of the calves spent more than a minute in the first contact with the brush. It is indicated in previous studies that the time healthy calves or cows spend on the brush increases with the time they are in the enriched environment (Toaff-Rosestein et al., 2017; Zobel et al., 2017).

Moreover, in adult cows the usage of automated brush has been linked to the detection of diseases (Mandel et al., 2017; Mandel et al., 2018), as grooming and, consequently, the use of automated brush are considered a low-resilience activity. Therefore, the usage decreased when energy resources are limited or in sickness or stress status when a reduction of activity is generated in order to save energy (Mandel et al., 2018). In addition, the use of automated brush in adult cows has been considered when describing behavior in peri-parturient cows (Newby et al., 2013) and in cows exposed to severe heat loads (Mandel et al., 2013). Newby et al. (2013) reported that cows housed with an automated brush spent more time licking their calves than cows housed without brushes. After calving, cows decreased the brush use. At separation, there was an increase in the use of the brush probably to cope with stress. Mandel et al. (2013) found that when food was located away from the brush, grooming activity was reduced. Also, a decrease in 50% of the use

was observed in the days of artificial insemination in comparison with previous days. Similarly, a reduction was observed with 1 unit increase of temperature and humidity index.

Research is encouraged to determine how disease, weather and longer periods of time might affect the use of grooming devices by dairy calves.

Conclusions

Our findings suggest that providing an automated brush to recently weaned calves housed in groups had an effect reducing the inactive time and increasing the eating and high active time. Our results did not show an effect on ADG or on presentation of diseases, even though our AB group had a smaller number of sick calves. From this study, we were able to describe behavioral patterns during the day and the positive interaction with the automated brush. Our study was conducted just in one rearing facility and the results represent the routines and managements of this specific organic farm. Further research is encouraged to assess behavioral effects of enriched environments on weaned dairy calves.

Tables and Figures

Table 3.1: Least square means (SE) for weaning weight, final weight, weight gain and ADG for treatment group.

Variable	AB	CON	P-Value
Weaning weight(Kg)	104.0 ± 1.24	103.3 ± 1.24	0.69
Final weight (Kg)	127.1 ± 1.71	125.3 ± 1.80	0.47
Weight gain (Kg)	21.9 ± 0.97	21.6 ± 1.02	0.79
ADG (Kg)	0.77 ± 0.03	0.75 ± 0.03	0.69

Table 3.2: Frequency of animals leaving the study due to disease by enrollment group during the 20 days in collective housing (P=0.12)

	Enrollment group				
Group	1	2	3	4	Total
CON, n (%)	5 (20)	4 (16)	4 (16)	3 (12)	16 (64)
AB, n (%)	5 (20)	1 (4)	3 (12)	0 (0)	9 (36)
Total, n (%)	10 (40)	5 (20)	7 (28)	3 (12)	25 (100)

Table 3.3: Results for the multivariate logistic regression for calves removed due to disease by treatment group and enrollment group (EG).

Group	Odds Ratio	95% CI	P-Value
CON vs AB	2.01	0.82 - 4.92	0.12
EG1 vs EG4	3.62	0.91- 14.42	0.09
EG2 vs EG4	1.8	0.29 - 8.21	0.77
EG3 vs EG4	2.59	0.61 - 10.92	0.51

Table 3.4: Frequency of calves that were treated for disease at least one time per enrollment and treatment group, as indicated by farm records (P=0.064).

	Enrollment group				
Group	1	2	3	4	Total
CON, n (%)	6 (21)	4(14)	5 (17)	4 (14)	19 (66)
AB, n (%)	5 (17)	2 (7)	3 (10)	0 (0)	10 (34)
Total, n (%)	11 (38)	6 (21)	8 (27)	4 (14)	29 (100)

Table 3.5: Frequency of calves that were reported with at least one health alarm per enrollment group, as indicated by the 3D accelerometer sensors (P= 0.36).

	Enrollment group				
Group	1	2	3	4	Total
CON, n (%)	12 (10)	17 (15)	17 (15)	15 (12)	61 (52)
AB, n (%)	13 (11)	14 (12)	14 (12)	15 (13)	56 (48)
Total, n (%)	25 (21)	31 (27)	31 (27)	30 (25)	117 (100)

Table 3.6: Frequency of calves that were reported with at least one very sick alarm per enrollment group, as indicated by the 3D accelerometer sensors (P=0.49).

	Enrollment group				
Group	1	2	3	4	Total
CON, n (%)	8 (9)	17 (18)	14 (15)	10 (10)	49 (52)
AB, n (%)	11 (11)	11 (12)	11 (12)	12 (13)	45 (48)
Total, n (%)	19 (20)	28 (30)	25 (27)	22 (23)	94 (100)

Table 3.7: Frequency of health alarms reported per calf and treatment group, as indicated by the 3D accelerometer sensors (P=0.44).

Group	Number of alarms					
	0	1	2	3	4	Total
CON, n (%)	21 (13)	23 (14)	28 (17)	7 (5)	2 (1)	81 (50)
AB, n (%)	25 (15)	26 (16)	22(14)	5 (3)	3 (2)	81 (50)
Total, n (%)	46 (28)	49 (30)	50 (31)	12 (8)	5 (3)	162 (100)

Table 3.8: Lapse of time to the first interaction of the calves with the automated brush (h:min), after being transferred to collective housing (first and last calf using the brush for the first time, mean and median of use by enrollment group).

Group	N	First calf	Last calf	Mean(SD)	Median
1	21	0:10	7:12	2:51(2:07)	2:10
2	20	0:09	38:54	6:57(9:50)	3:43
3	22	0:05	65:53	12:05(16:59)	7:45

Table 3.9: Frequency of calves by duration of use of the automated brush in the first interaction (categorized as < 1 minute, ≥ 1 minute) by enrollment group.

	Group				
Time in use	1	2	3	Total	%
< 1 min	9	6	12	27	43
≥ 1 min	12	14	10	36	57
Total	21	20	22	63	100

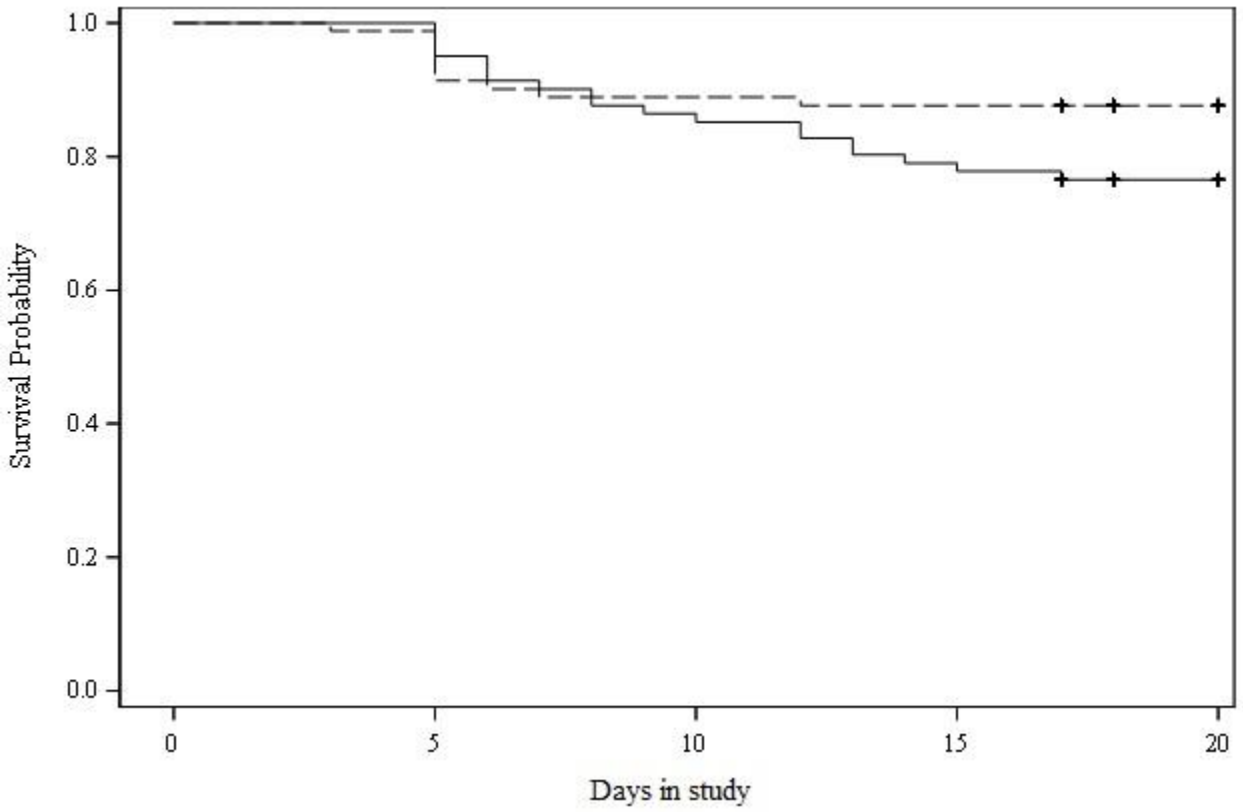

Figure 3.1: Survival curve comparing the time to first disease detected by farm personnel (P=0.12) for control group (solid line) and automated brush group (dashed line).

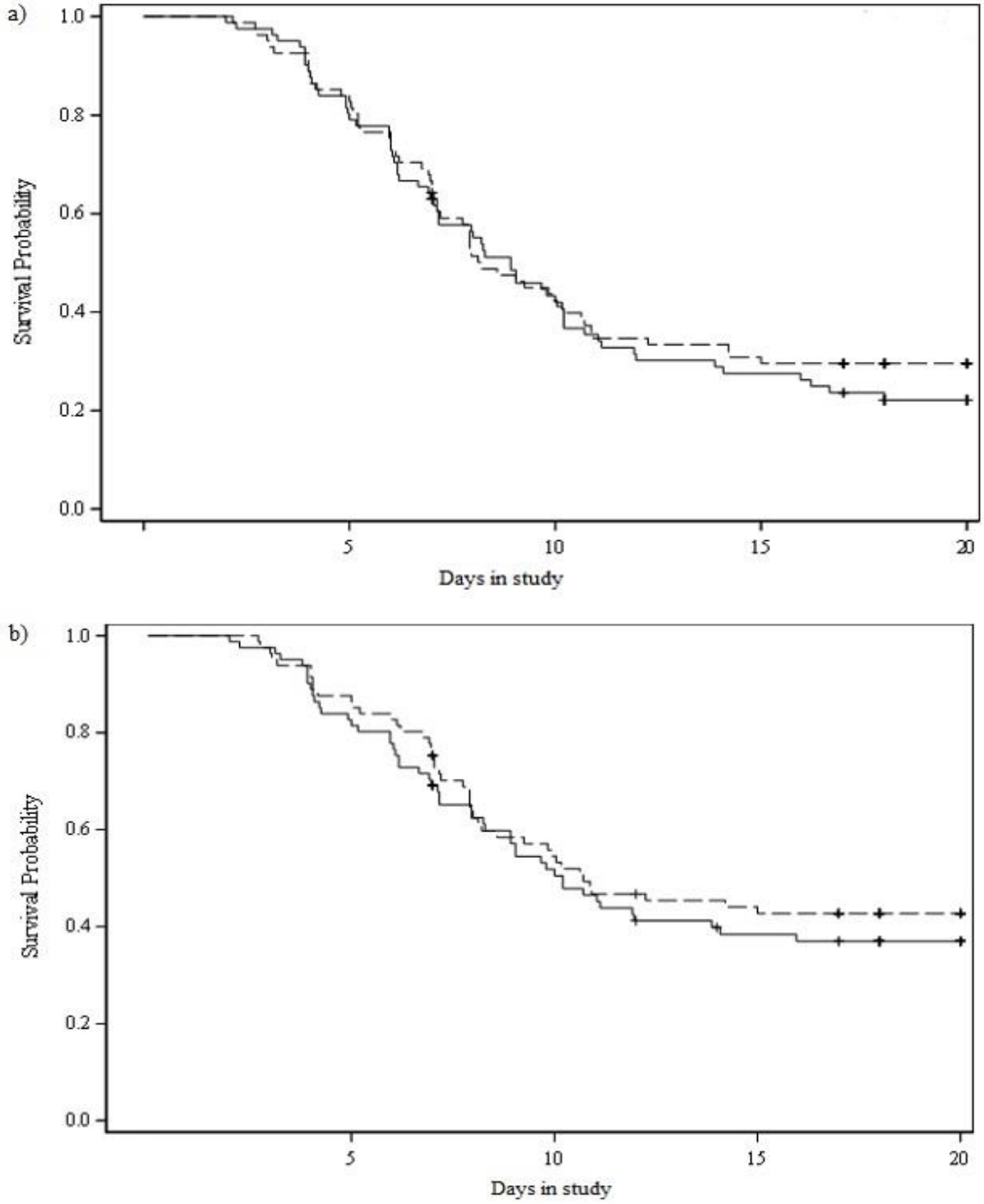

Figure 3.2: Comparison of time to first health alarm by 3D accelerometer sensors (P=0.77) for control (solid line) and automated brush group (dashed line); a). Comparison of time to a first very sick alarm (P=0.48) for control group (solid line) and automated group (dashed line); b).

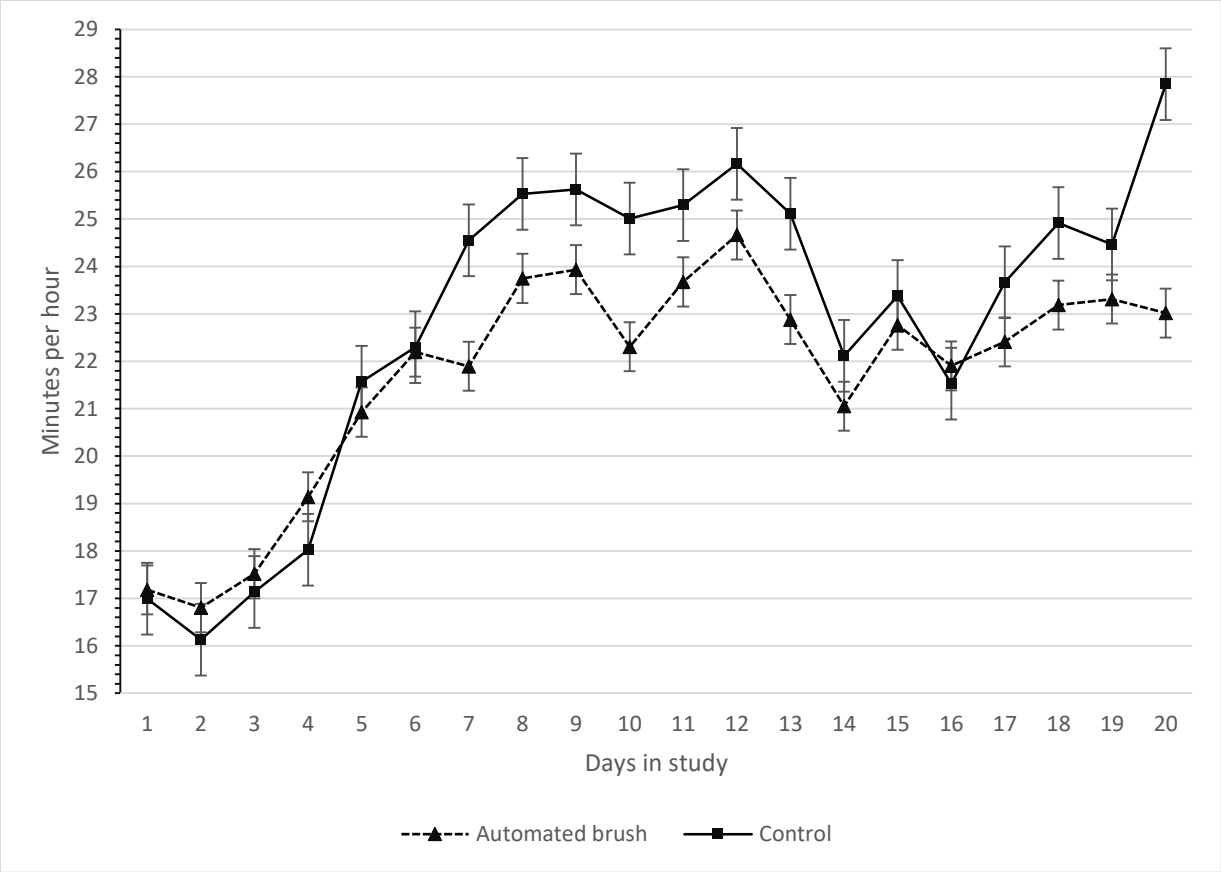

Figure 3.3: Average "not-active" time[1] (minutes per hour) spent for CON (solid line) and AB (dotted line) group per day in study (P=0.014).

[1] Not-active time: total time per hour than calves were not: eating, ruminating, active or high active.

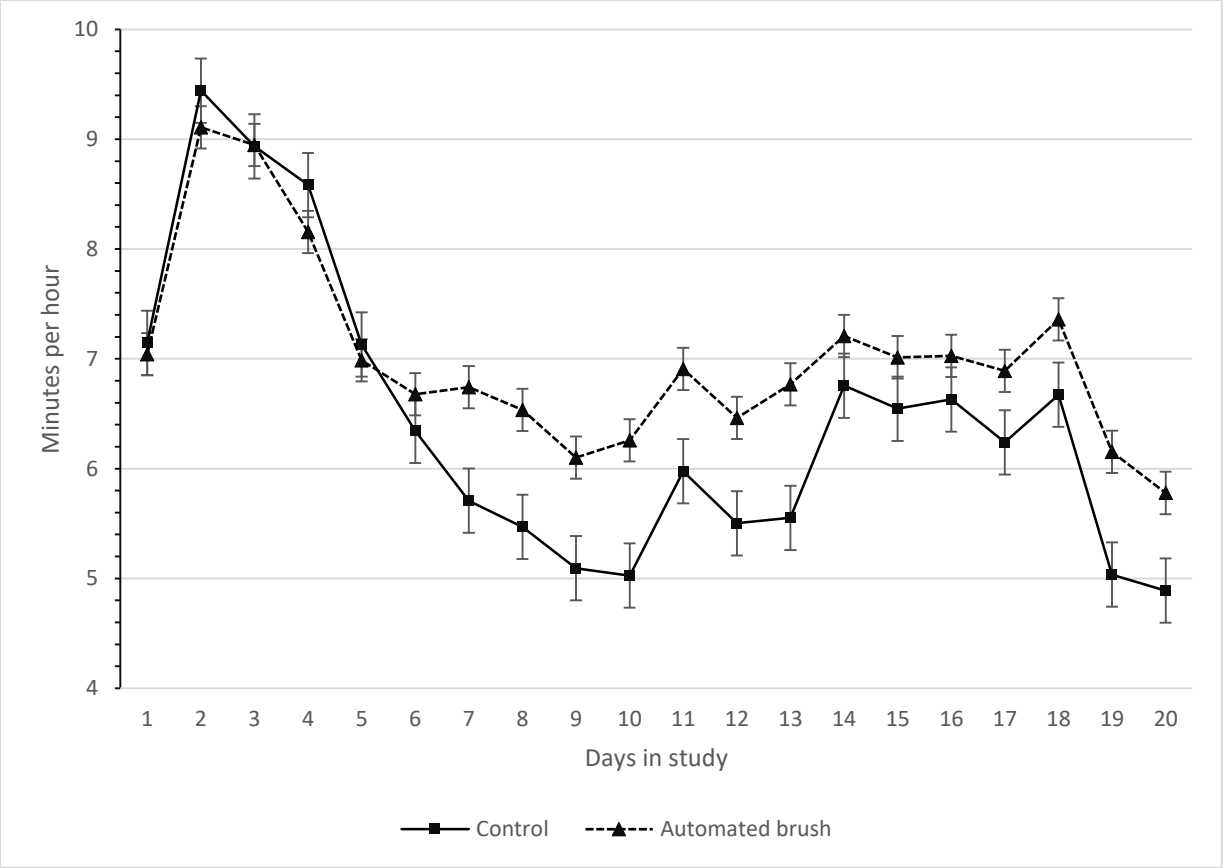

Figure 3.4: Average "eating" time (minutes per hour) spent for control (solid line) and automated brush (dotted line) group per day in study (P=0.012).

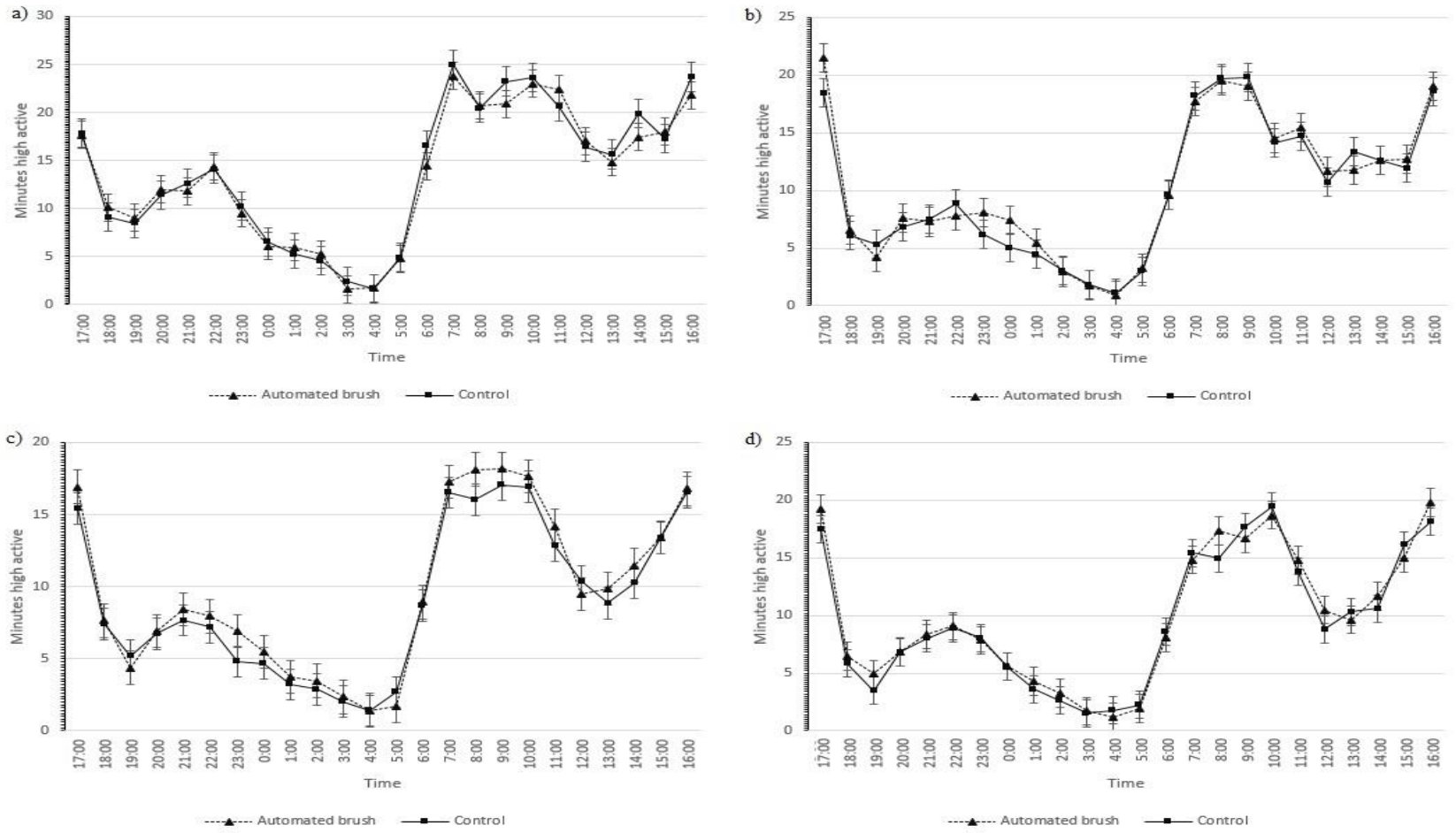

Figure 3.5: Average "high active" time (minutes) spent by hour for control (solid line) and automated brush (dotted line) group after transfer to collective housing. Period 1 (day 1 to day 5, P<0.0001); a). Period 2 (day 6 to 10 in study, P<0.0001); b). Period 3 (day 11 to 15 in study, P= 0.049); c). Period 4 (day 16 to 20 in study, P= 0.02); d).

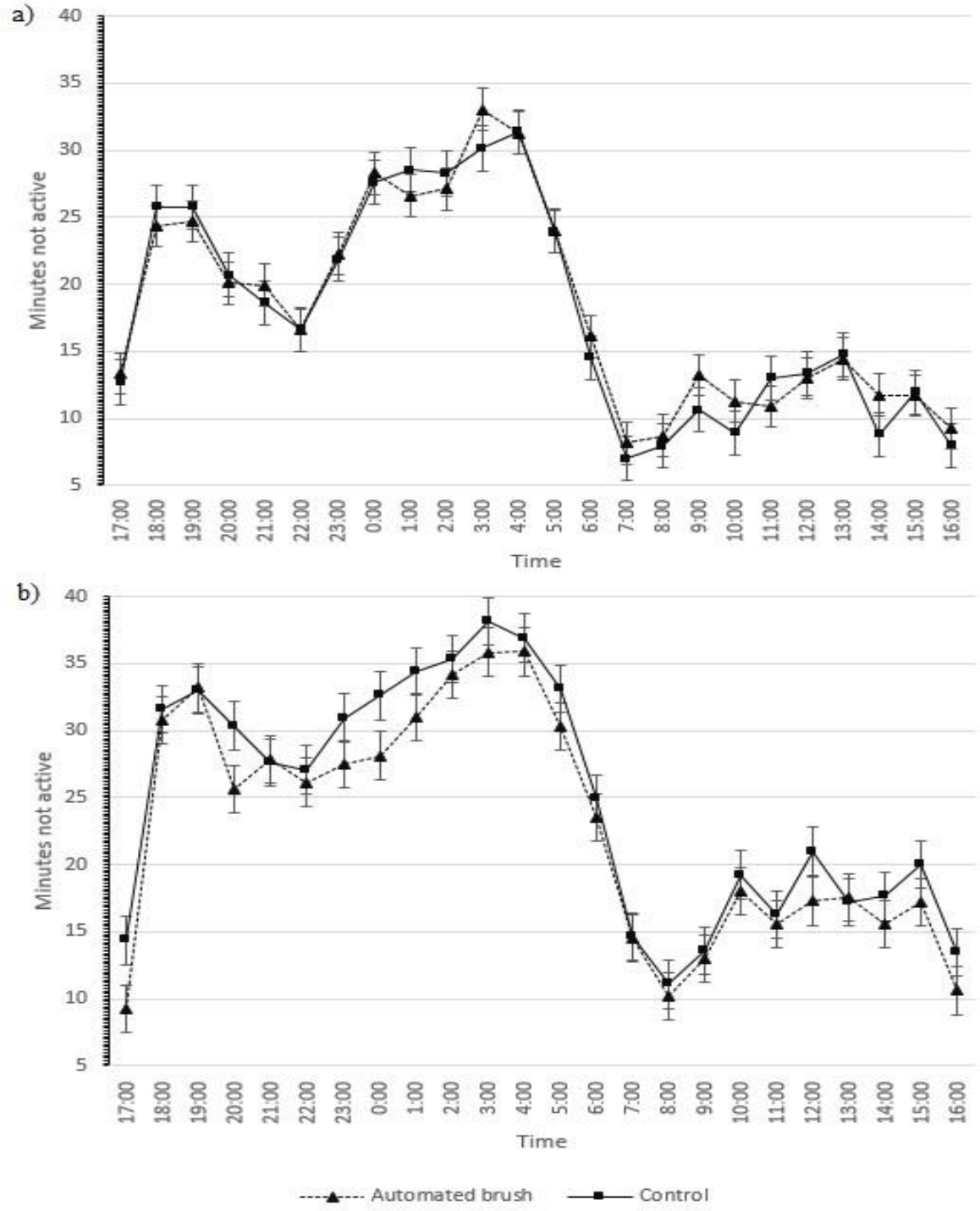

Figure 3.6: Average "not-active" time[1] (minutes) spent by hour for control (solid line) and automated brush (dotted line) group after transfer to collective housing. Period 1 (day 1 to day 5, P= 0.0002); a). Period 2 (day 6 to 10 in study, P=0.003); b)

[1] Not-active time: total time per hour than calves were not: eating, ruminating, active or high active

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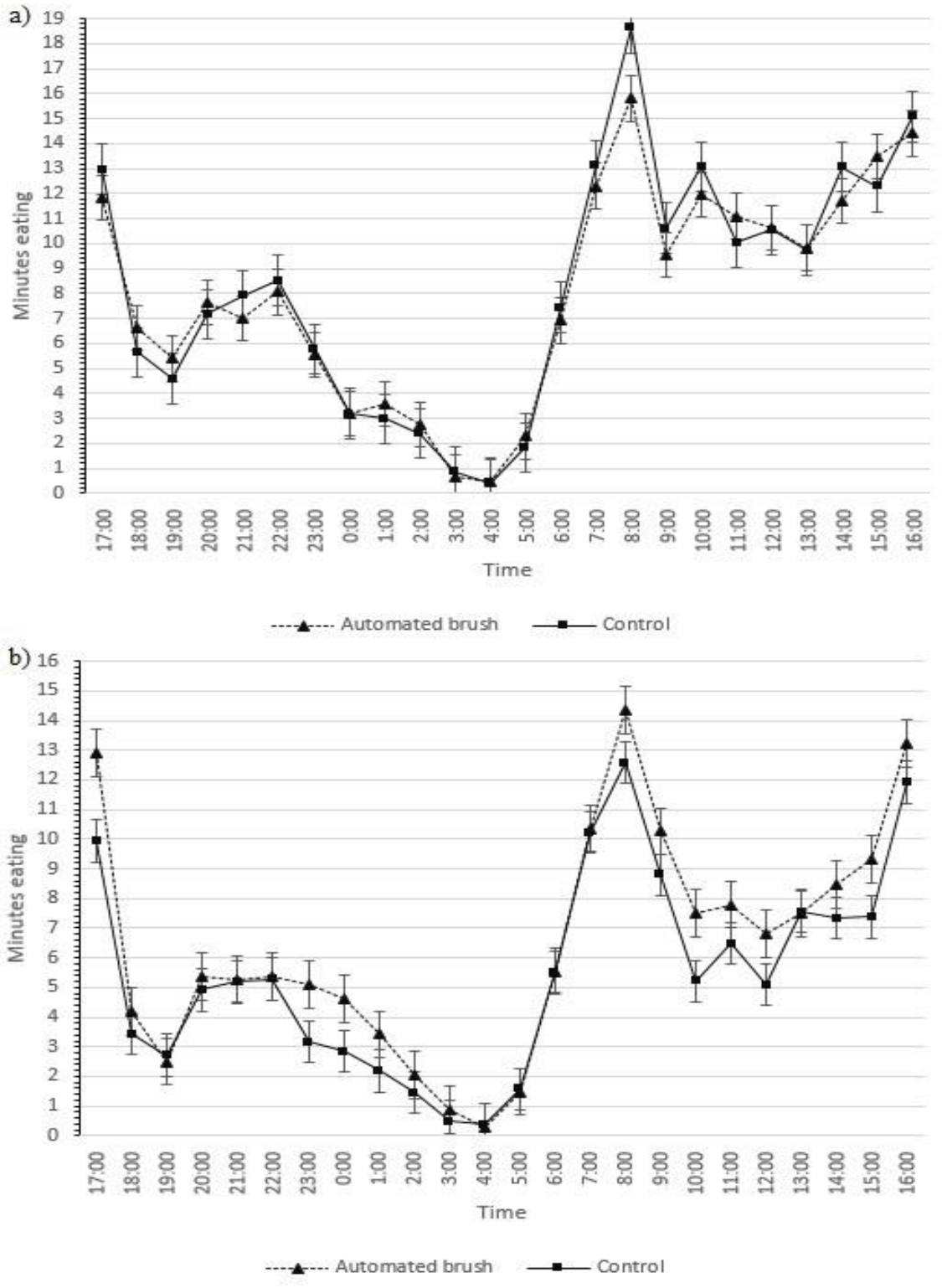

Figure 3.7: Average "eating" time (minutes) spent by hour for control (solid line) and automated brush (dotted line) group after transfer to collective housing. Period 1 (day 1 to day 5, P=0.0002); a). Period 2 (day 6 to 10 in study, P= 0.003); b).

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CHAPTER 4: GENERAL CONCLUSIONS

This thesis had the objective of exploring alternatives to enhance health and well-being of dairy calves. From chapter 2, SRB did not represent an efficient alternative for the prevention or treatment of neonatal diarrhea after being added in the milk of pre-weaned calves. Additionally no effects on immunity or performance were observed. One of the main challenges in this study was the proper suspension of SRB in milk. Research with SRB as a prebiotic in older animals and in other forms of presentation that allow reduce the daily dose provided to the animals is encouraged.

From chapter 3, weaned calves spent more time “eating” and less time “not active” than control calves when an automated grooming brush was provided in collective pens, although no other differences were found. Research with automated grooming devices for longer periods of time in pens of recently weaned calves in order to stablish a better understanding of long term effects on behavior, performance and health is advised.

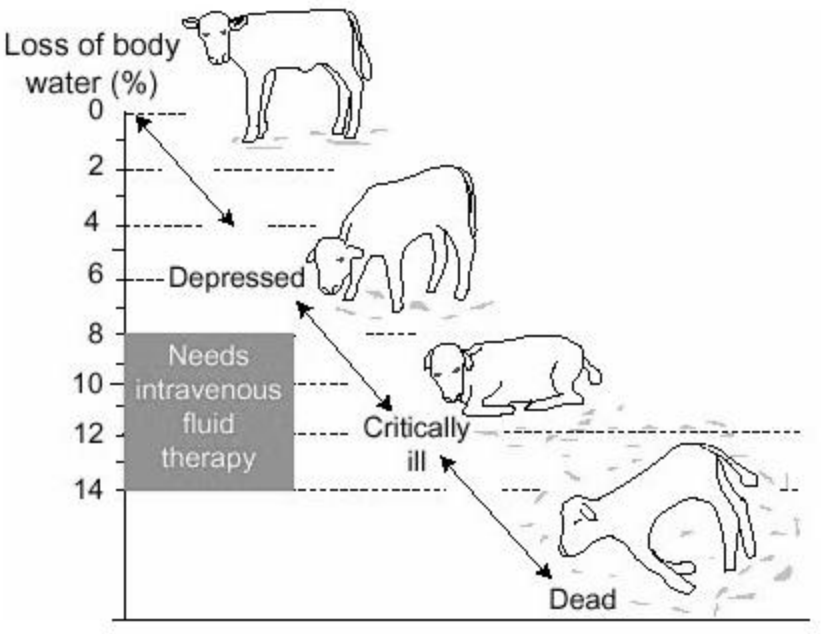
APPENDIX

A2.1: Nutritional composition of the organic calf starter provided to pre-weaned calves. Guaranteed analysis by manufacturer (Feedex Companies, South Hutchinson, KS)

Nutrient	Concentration
CP (%)	16
Fat (%)	2.4
Fiber (%)	3
ADF (%)	5
Ca (%)	0.7 - 1.2
P (%)	0.45
NaCl (%)	0.2 - 0.7
Mg (%)	0.2
K (%)	0.9
Cu (ppm)	15
Se (ppm)	0.3
Zn (ppm)	80
Vitamin A (IU/lb)	5000
Vitamin D (IU/lb)	1000

A2.2: Dehydration percentage in calves and clinical signs of disease. Adaptation from Wattiaux, 2005.

Dehydration	Clinical Signs
5 – 6 %	Diarrhea, no clinical signs, strong suckle reflex.
6 – 8 %	Mild depression, skin tent 2 to 6 seconds, sunken eyes, weak suckle reflex.
8 – 10 %	Calf depressed, laying down, very sunken eyes, dry gums, skin tent >6 s.
10 – 14 %	Calf will not stand, cold extremities, skin tent persist, comatose.
> 14 %	Death



A2.3: Dehydration percentage in calves and visual representation of clinical signs. Adaptation from Wattiaux, 2005.

A.3.1: Nutritional composition of the TMR offered to weaned calves. Analysis provided by farm management.

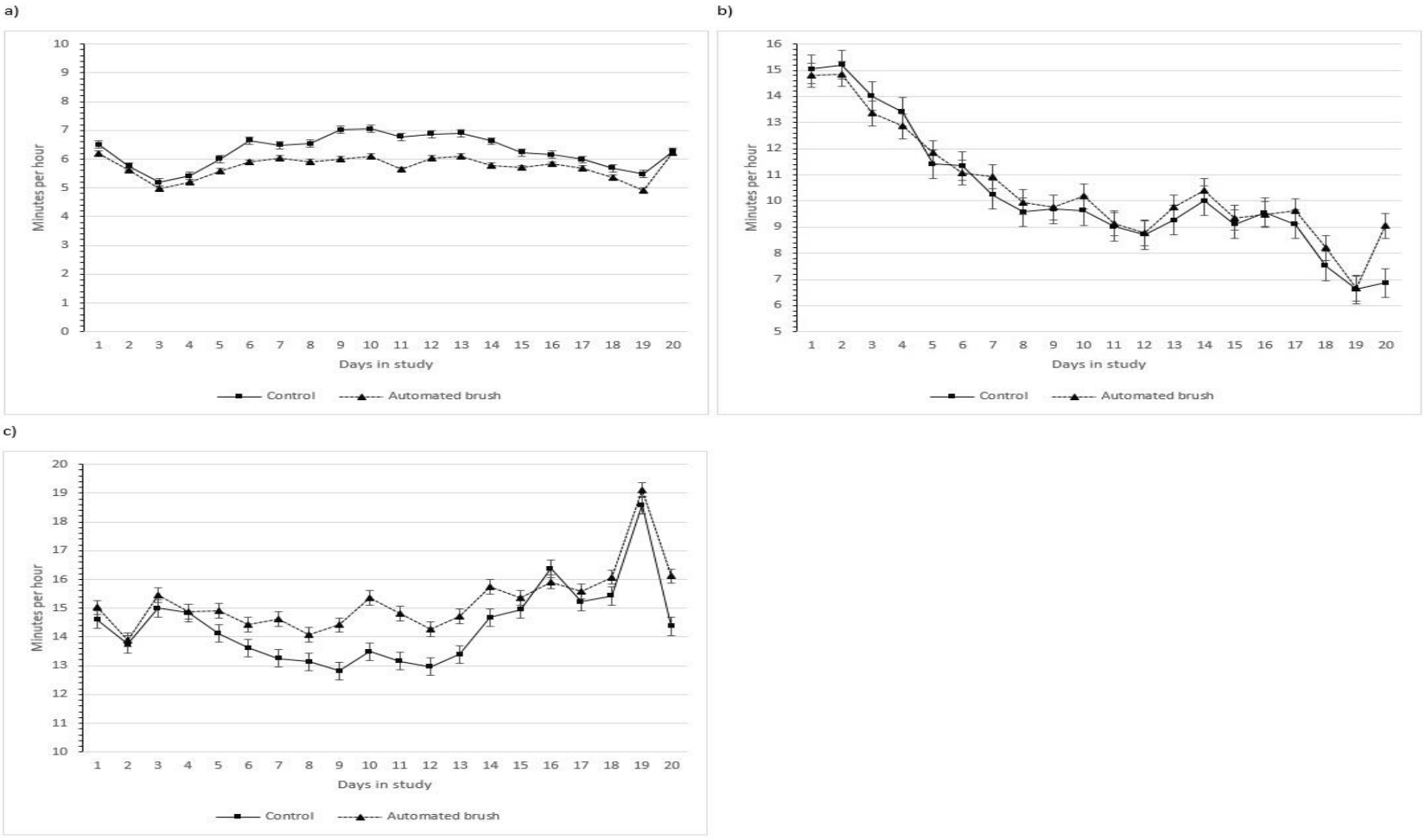
Nutrient	Concentration
Moisture (%)	53.3
Dry Matter (%)	46.7
Crude Protein (%DM)	16.2
Soluble Protein (%DM)	5.2
ADF Protein (ADICP) (%DM)	0.92
NDF Protein (NDICP) (%DM)	1.35
ADF (%DM)	26.4
Crude Fat (%DM)	4.31
Ash (%DM)	8.68
Calcium (%DM)	1.02
Phosphorus (%DM)	0.35
Magnesium (%DM)	0.44
Potassium (%DM)	1.83
Sodium (%DM)	0.26
Iron (PPM)	355
Manganese (PPM)	92
Zinc (PPM)	111
Copper (PPM)	24
TDN (%DM)	68.1
Net Energy Lactation (mcal/lb)	0.7
Net Energy Maintenance (mcal/lb)	0.7
Net Energy Gain (mcal/lb)	0.43
Non Fiber Carbohydrates (%DM)	37.4

A3.2: Least square means (SE) for "not-active" time (minutes per hour) spend by control and automated brush groups by day.

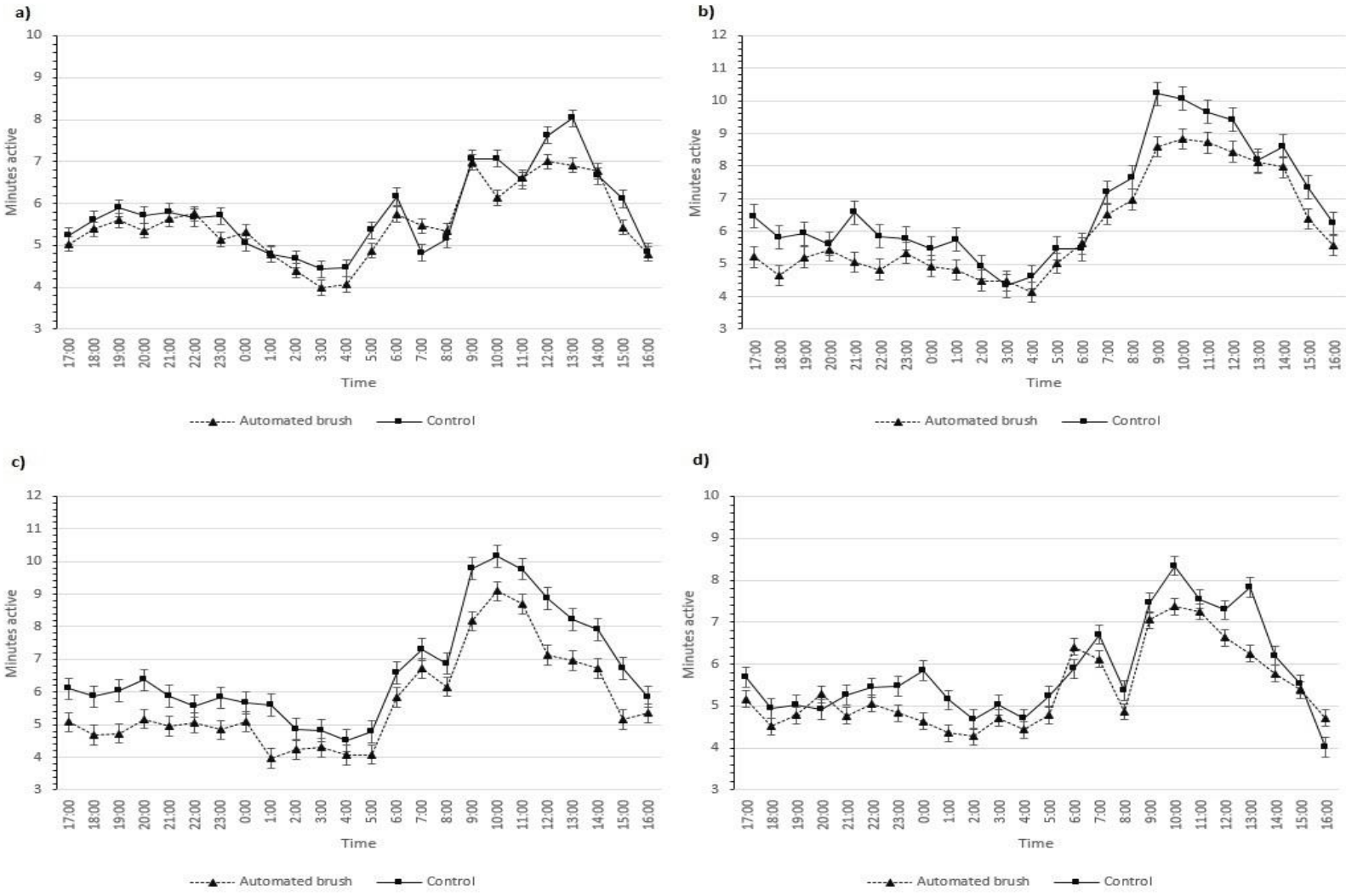
Day	A. Brush	SE	Control	SE	P-value
1	17.1	0.76	17.0	0.76	<.0001
2	16.8	0.76	16.1	0.76	<.0001
3	17.5	0.76	17.1	0.76	<.0001
4	19.1	0.76	18.0	0.76	<.0001
5	20.9	0.76	21.6	0.76	<.0001
6	22.2	0.76	22.3	0.76	<.0001
7	21.9	0.76	24.5	0.76	<.0001
8	23.7	0.76	25.5	0.76	<.0001
9	23.9	0.78	25.6	0.78	<.0001
10	22.3	0.78	25.0	0.78	<.0001
11	23.7	0.78	25.3	0.78	<.0001
12	24.6	0.78	26.2	0.78	<.0001
13	22.9	0.78	25.1	0.78	<.0001
14	21.1	0.79	22.1	0.80	<.0001
15	22.8	0.79	23.4	0.81	<.0001
16	21.9	0.79	21.5	0.82	<.0001
17	22.4	0.79	23.7	0.82	<.0001
18	23.2	0.80	24.9	0.82	<.0001
19	23.3	0.88	24.5	0.91	<.0001
20	23.0	1.41	27.8	1.41	<.0001

A3.3: Least square means (SE) for "eating" time (minutes per hour) spend by control and automated brush groups by day.

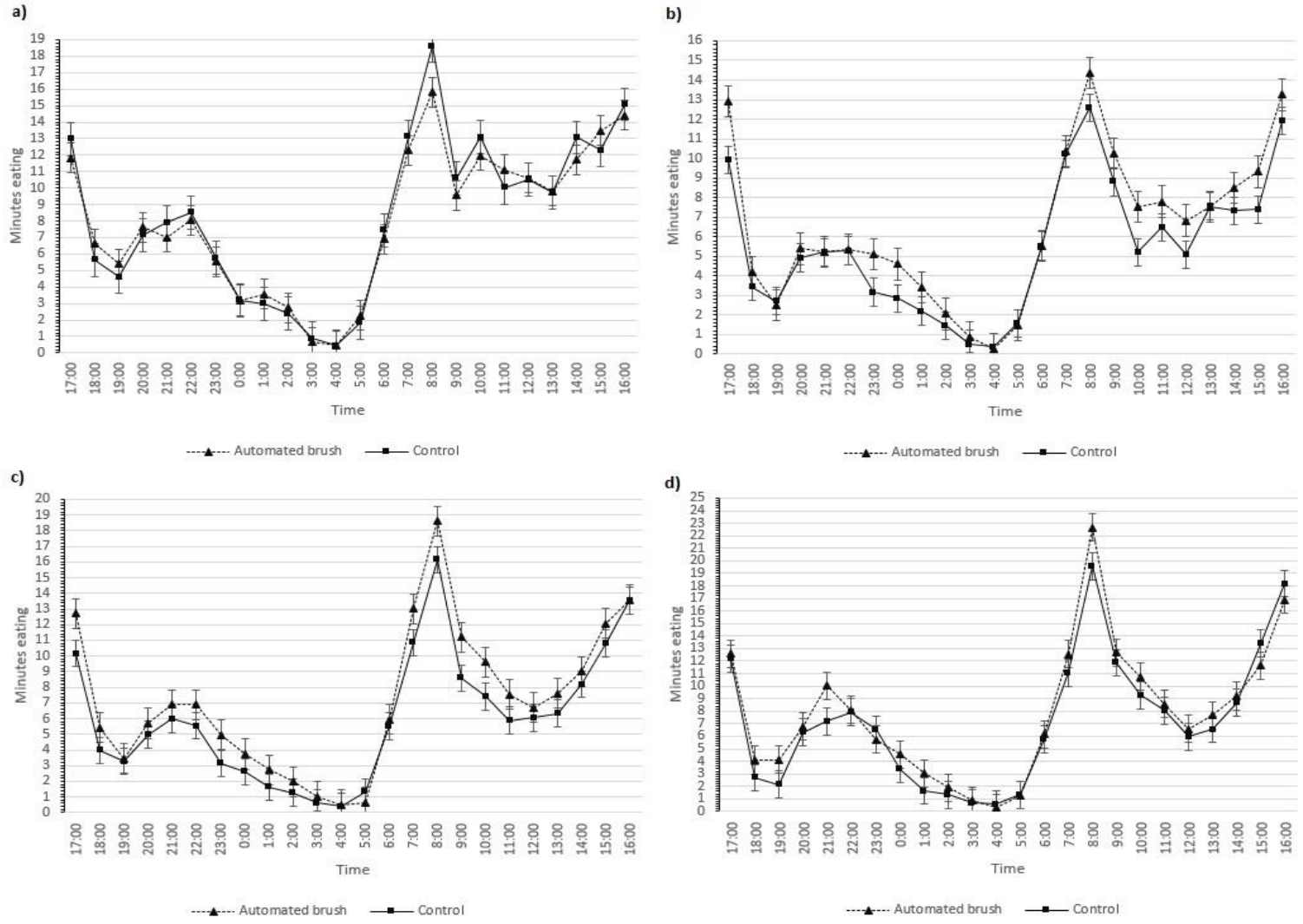
Day	A. Brush	SE	Control	SE	P-Value
1	7.0	0.38	7.1	0.38	<.0001
2	9.1	0.38	9.4	0.38	<.0001
3	8.9	0.38	8.9	0.38	<.0001
4	8.2	0.38	8.5	0.38	<.0001
5	6.9	0.38	7.1	0.38	<.0001
6	6.7	0.38	6.3	0.38	<.0001
7	6.7	0.38	5.7	0.38	<.0001
8	6.5	0.38	5.5	0.38	<.0001
9	6.1	0.39	5.1	0.39	<.0001
10	6.3	0.39	5.0	0.39	<.0001
11	6.9	0.39	5.9	0.39	<.0001
12	6.5	0.39	5.5	0.39	<.0001
13	6.8	0.39	5.6	0.39	<.0001
14	7.2	0.39	6.8	0.39	<.0001
15	7.0	0.39	6.5	0.40	<.0001
16	7.0	0.39	6.6	0.40	<.0001
17	6.9	0.39	6.2	0.40	<.0001
18	7.4	0.39	6.7	0.40	<.0001
19	6.2	0.43	5.0	0.44	<.0001
20	5.8	0.65	4.9	0.65	<.0001



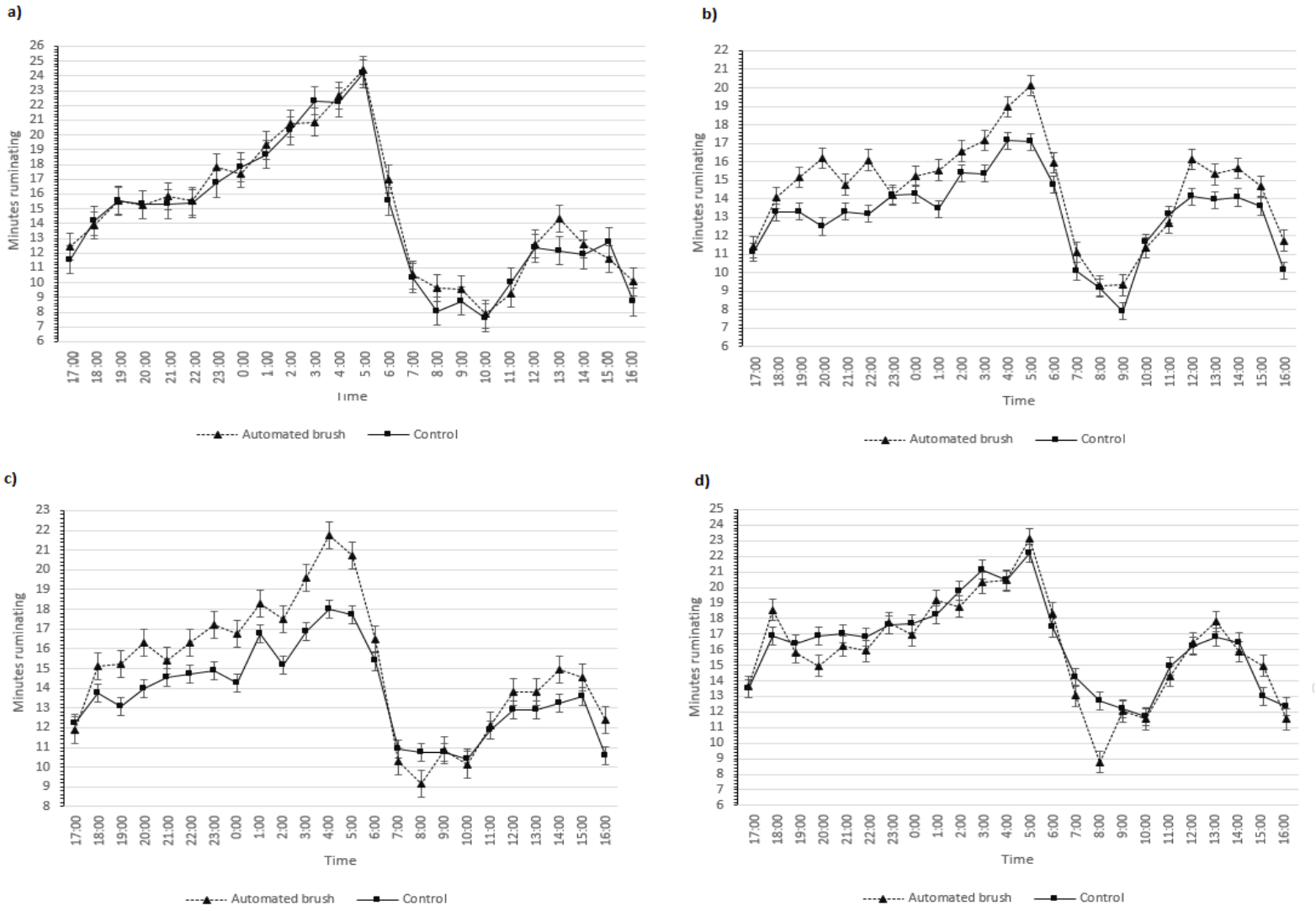
A3.4: Average "active" time (minutes per hour) spent for control (solid line) and automated brush (dotted line) group per day in study (P=0.36); a). Average "high active" time (minutes per hour) spent for control (solid line) and automated brush (dotted line) group per day in study (P=0.12); b). Average "ruminating" time (minutes per hour) spent for control (solid line) and automated brush (dotted line) group per day in study (P=0.28); c).



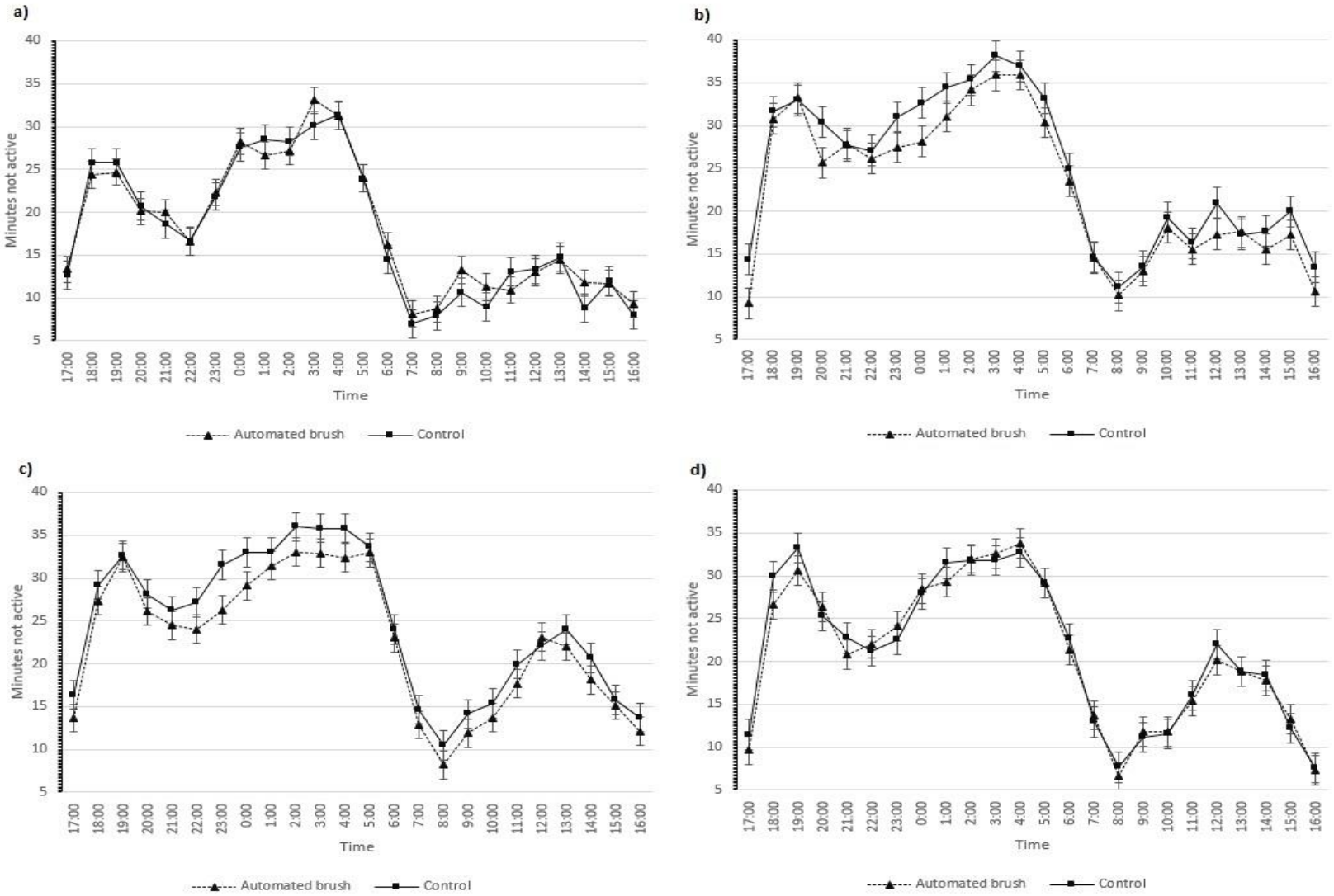
A3.5: Average "active" time (minutes) spent by hour for control (solid line) and automated brush (dotted line) group after transfer to collective housing. Period 1 (day 1 to day 5); a). Period 2 (day 6 to 10); b). Period 3 (day 11 to 15); c). Period 4 (day 16 to 20); d).



A3.6: Average "eating" time (minutes) spent by hour for control (solid line) and automated brush (dotted line) group after transfer to collective housing. Period 1 (day 1 to day 5); a). Period 2 (day 6 to 10); b). Period 3 (day 11 to 15); c). Period 4 (day 16 to 20); d).



A3.7: Average "ruminating" time (minutes) spent by hour for control (solid line) and automated brush (dotted line) group after transfer to collective housing. Period 1 (day 1 to day 5); a). Period 2 (day 6 to 10); b). Period 3 (day 11 to 15); c). Period 4 (day 16 to 20); d).



A3.8: Average "not-active" time (minutes) spent by hour for control (solid line) and automated brush (dotted line) group after transfer to collective housing. Period 1 (day 1 to day 5); a). Period 2 (day 6 to 10); b). Period 3 (day 11 to 15); c). Period 4 (day 16 to 20); d).