EFFECT OF HOUSING AND FEEDING SYSTEM FACTORS ON BOVINE WELFARE

LOURDES LLONCH FERNÁNDEZ

Universitat Autònoma de Barcelona

EFFECT OF HOUSING AND FEEDING SYSTEM FACTORS ON BOVINE WELFARE

Tesis doctoral presentada per

LOURDES LLONCH FERNÁNDEZ

Dirigida per

ALFRED FERRET QUESADA

LORENA CASTILLEJOS VELÁZQUEZ

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Alfred Ferret Quesada i **Lorena Castillejos Velázquez**, investigadors del Departament de Ciència Animal i dels Aliments de la Facultat de Veterinària de la Universitat Autònoma de Barcelona,

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Que la memòria titulada **"Effect of housing and feeding system factors on bovine welfare"**, presentada per Lourdes Llonch Fernández amb la finalitat d'optar al grau de Doctor en Veterinària, ha estat realitzada sota la seva direcció i, considerant-la acabada, autoritzen la seva presentació per que sigui jutjada per la comissió corresponent.

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Dr. Alfred Ferret Quesada

Dra. Lorena Castillejos Velázquez

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I was taught that the way of progress is neither swift nor easy

Marie Curie

De ben petits ens ensenyen que la paraula “gràcies” és màgica, i després d’uns quants anys sense que em preguntin “Què es diu?”, crec fermament que aquesta paraula té poders. Només cal posar-la en pràctica i comprovar-ho, a més... és gratis utilitzar-la. I per això mateix aquí estic, escrivint aquestes línies per agrair a tots els que han contribuït, bé amb un granet de sorra o amb cubells i camions d’ella, a aconseguir tancar una etapa de la meva vida amb aquesta tesis doctoral com a prova.

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SUMMARY

The farm management strategies in the cattle production system can improve animal welfare as well as other factors related to the production system. In this doctoral thesis, the strategies studied were a feeding system in beef cattle and a housing system in dairy cows, which modify farm resources or management to meet animal needs.

In the first study, the objective was to establish the optimal physical effective fiber (peNDF) proportion in high-concentrate diets fed to beef cattle, to reduce the risk of subacute ruminal acidosis. Simmental heifers in experimental conditions were fed 4 diets with different peNDF proportions: 6.4%, 10.4%, 13.6%, and 15.4%, offered ad libitum as total mixed ration, and containing 15% barley straw and 85% concentrate. Records about intake, intake by particle size, feed sorting, behavioral activities, and rumen pH provided insight on the proposed objective. The results suggested that the 10.4% diet best met the requirements of not compromising intake, limiting sorting behavior, and promoting time spent ruminating to reduce the number of hours under rumen pH thresholds. In addition, dietary peNDF has to be increased in cattle diets because it stimulates rumination activity, but it must be done at a level which does not reduce intake or lead to sorting against large particles.

In the second study, the objective was to compare forest biomass to sawdust as bedding material for compost-bedded pack (CBP) barns on CBP performance, bedding microbial counts, and welfare of nonlactating cows. Holstein cows in experimental conditions were allocated on a CBP with sawdust (CBP-S) or forest biomass (CBP-FB). The results of CBP temperature, moisture and C:N ratio showed that CBP performance was worse in CBP-FB than in CBP-S. In addition, cow comfort was worse in CBP-FB than in CBP-S considering the results obtained for time needed to lie down. However, bedding microbial counts showed that some microbial species were better controlled in CBP-FB than in CBP-S. Nevertheless, although forest biomass appeared to support a composting process and controlled the temporal evolution of bedding microbial counts of some species, higher required volumes of forest biomass and market prices of materials could have a greater economic impact on farm profitability.

In the third study, the objective was to ascertain the agronomic value of both materials resulting from the CBP. Once cows had been moved away, the CBP materials were used to build 2 composting piles with the further objective of ascertaining the agronomic characteristics of both materials after conducting an additional composting process. Some characteristics of chemical composition and granulometry of raw forest biomass made it a suitable bedding material to be used as CBP, but its high moisture content could limit the ability to absorb liquid manure. Taken together the degree of stability of the organic matter with the temperature evolution of CBP, this suggests that a real composting process did not occur in any material. The composting process of the piles did not lead to any relevant change in CBP materials, and only the organic matter of forest biomass pile was stabilized. From the agronomic point of view, sawdust and forest biomass presented potentially valuable characteristics as regards organic amendment in the soil, thanks to their high organic matter content and low nutrient content.

RESUM

Les estratègies de maneig en granges de producció bovina poden millorar el benestar animal, a més d’altres factors relacionats amb el sistema productiu. En aquesta tesi doctoral es van estudiar estratègies relacionades amb el sistema d'alimentació en boví de carn i el sistema d'allotjament en boví de llet, les quals van modificar els recursos o el maneig de la granja per cobrir les necessitats dels animals.

En el primer estudi, l'objectiu va ser establir la proporció òptima de fibra físicament efectiva (peNDF) en dietes altament concentrades de boví de carn, per reduir el risc d'acidosi ruminal subclínica. En condicions experimentals, vedelles de raça Simmental van ser alimentades amb 4 dietes amb diferent proporció de peNDF: 6,4%, 10,4%, 13,6%, i 15,4%, distribuïdes ad libitum com a ració mixta completa que conté 15% de palla d'ordi i 85% de concentrat. Els registres sobre ingesta, ingesta per grandària de partícula, selecció de l'aliment, activitats conductuals, i pH ruminal van donar informació de l’objectiu plantejat. Els resultats van suggerir que la dieta amb 10,4% de peNDF és la més adequada al no comprometre la ingesta, limitar la conducta de selecció, i promoure el temps dedicat a rumiar per reduir el nombre d'hores per sota els llindars de pH ruminal. A més, la peNDF de la dieta s'ha d'augmentar en les dietes de boví perquè estimula la rumia, però ha de fer-se a un nivell que no redueixi la ingesta ni permeti seleccionar en contra de partícules grans.

En el segon estudi, l'objectiu va ser comparar la biomassa forestal amb les serradures com a material de llit per estabulacions de llit compostat (CBP) sobre el rendiment de CBP, el recompte microbià del llit, i el benestar de vaques no lactants. En condicions experimentals, vaques de raça Holstein van ser allotjades en CBP amb serradures (CBP-S) o biomassa forestal (CBP-FB). Els resultats de temperatura, humitat

i relació C:N de CBP van demostrar que el rendiment de CBP va ser pitjor en CBP-FB que en CBP-S. Així mateix, el confort de les vaques va ser pitjor en CBP-FB que en CBP-S considerant els resultats del temps necessari per tombar-se. Però els recomptes microbians de CBP mostraren que algunes especies microbianes van ser millor controlades en CBP-FB que en CBP-S. No obstant, encara que la biomassa forestal va semblar promoure el procés de compostatge i va controlar l'evolució temporal d'alguns recomptes microbians de CBP, la demanda de volums majors de biomassa forestal i els preus de mercat dels materials podrien tenir un gran impacte econòmic en la rendibilitat de la granja.

En el tercer estudi, l'objectiu va ser determinar el valor agronòmic d'ambdós materials resultants de CBP. Quan les vaques van abandonar l'estabulació, els materials de CBP es van utilitzar per construir 2 piles de compostatge amb l'objectiu afegit de determinar les característiques agronòmiques d'ambdós materials després de realitzar un procés de compostatge addicional. Algunes característiques de la composició química i la granulometria de la biomassa forestal inicial la fan un material de llit adequat per utilitzar com CBP, però la seva alta humitat podria limitar la capacitat d'absorbir dejeccions. El grau d'estabilitat de la matèria orgànica amb l'evolució de la temperatura de CBP suggereixen que no es va produir un compostatge real en cap material. El procés de compostatge de les piles no va comportar cap canvi rellevant en els materials de CBP i només es va estabilitzar la matèria orgànica de la pila de biomassa forestal. Des del punt de vista agronòmic, serradures i biomassa forestal presentaven característiques potencialment valuoses com esmena orgànica del sòl pel seu contingut alt en matèria orgànica i baix en nutrients.

RESUMEN

Las estrategias de manejo en granjas de producción bovina pueden mejorar el bienestar animal, además de otros factores relacionados con el sistema productivo. En esta tesis doctoral se estudiaron estrategias relacionadas con el sistema de alimentación en vacuno de carne y el sistema de alojamiento en vacuno de leche, las cuales modificaron los recursos o el manejo de la granja para cubrir las necesidades de los animales.

En el primer estudio, el objetivo fue establecer la proporción óptima de fibra físicamente efectiva (peNDF) en dietas altamente concentradas de vacuno de carne, para reducir el riesgo de acidosis ruminal subclínica. En condiciones experimentales, terneras de raza Simmental fueron alimentadas con 4 dietas con diferente proporción de peNDF: 6,4%, 10,4%, 13,6%, y 15,4%, ofrecidas ad libitum como ración mixta completa que contiene 15% de paja de cebada y 85% de concentrado. Los registros sobre ingesta, ingesta por tamaño de partícula, selección del alimento, actividades conductuales, y pH ruminal dieron información del objetivo planteado. Los resultados sugirieron que la dieta de 10,4% es la más adecuada al no comprometer la ingesta, limitar la conducta de selección, y promover el tiempo dedicado a rumiar para reducir el número de horas por debajo los umbrales de pH ruminal. Además, la peNDF de la dieta se tiene que aumentar en las dietas de vacuno porque estimula la rumia, pero tiene que hacerse a un nivel que no reduzca la ingesta ni permita seleccionar en contra de partículas grandes.

En el segundo estudio, el objetivo fue comparar la biomasa forestal con el serrín como material de cama para estabulaciones de cama compostada (CBP) sobre el rendimiento de CBP, el recuento microbiano de la cama, y el bienestar de vacas no lactantes. En condiciones experimentales, vacas de raza Holstein fueron alojadas en CBP con serrín (CBP-S) o biomasa forestal (CBP-FB). Los resultados de temperatura, humedad y relación C:N de CBP demostraron que el rendimiento de CBP fue peor en CBP-FB que en CBP-S. Asimismo, el confort de las vacas fue peor en CBP-FB que en CBP-S considerando los resultados del tiempo necesario para tumbarse. Pero los recuentos microbianos de CBP mostraron que algunas especies microbianas fueron mejor controladas en CBP-FB que en CBP-S. No obstante, aunque que la biomasa forestal pareció promover el proceso de compostaje y controló la evolución temporal de algunos recuentos microbianos de CBP, la demanda de volúmenes mayores de biomasa forestal y los precios de mercado de los materiales podrían tener un gran impacto económico en la rentabilidad de la granja.

En el tercer estudio, el objetivo fue determinar el valor agronómico de ambos materiales resultantes de CBP. Cuando las vacas abandonaron la estabulación, los materiales de CBP se utilizaron para construir 2 pilas de compostaje con el objetivo añadido de determinar las características agronómicas de ambos materiales después de realizar un proceso de compostaje adicional. Algunas características de la composición química y la granulometría de la biomasa forestal inicial la hacen un material de cama adecuado para utilizar como CBP, pero su alta humedad podría limitar la capacidad de absorber deyecciones. El grado de estabilidad de la materia orgánica con la evolución de la temperatura de CBP sugieren que no se produjo un compostaje real en ningún material. El proceso de compostaje de las pilas no conllevó ningún cambio relevante en los materiales de CBP y sólo se estabilizó la materia orgánica de la pila de biomasa forestal. Desde el punto de vista agronómico, serrín y biomasa forestal presentaban características potencialmente valiosas como enmienda orgánica del suelo por su contenido alto en materia orgánica y bajo en nutrientes.

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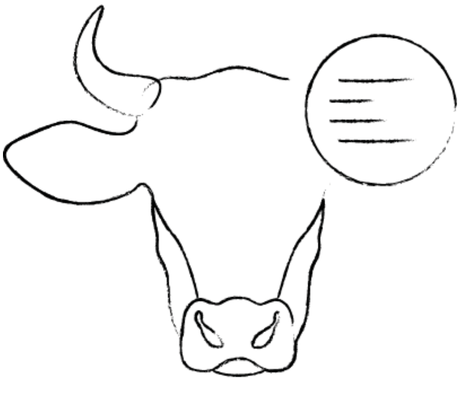
ABBREVIATIONS

ADF	Acid detergent fiber
ADG	Average daily gain
AUC	Area under the curve
BCS	Body condition score
BW	Body weight
C	Carbon
CBP	Compost-bedded pack
CBP-FB	Compost-bedded pack with forest biomass
CBP-S	Compost-bedded pack with sawdust
Cp	Conceptual predictive criteria
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
EC	Electric conductivity
EE	Ether extract
eNDF	Effective neutral detergent fiber
FB	Forest biomass
K	Potassium
N	Nitrogen
NDF	Neutral detergent fiber
OM	Organic matter
P	Phosphorus
pef	Physical effectiveness factor
peNDF	Physically effective neutral detergent fiber
PRESS	Predicted residual error sum of squares
PSPS	Penn state particle separator
$\mathbf{R^2}$	Determination coefficient
RMSE	Root mean square error
S	Sawdust
SD	Stability degree
SARA	Subacute ruminal acidosis
SCC	Somatic cell count
SCFA	Short-chain fatty acid
TBC	Total bacteria count
TMR	Total mixed ration
VIF	Variance inflation factor
W	Week



CHAPTER 1.

General Introduction

1.1. The Cattle Industry

The cattle industry in Spain has undergone a big change in recent decades. Basically, there has been a shift from little herds managed by families, with heterogeneous milk and meat production and a feeding system based on pasture availability, to a specialized dual system, between dairy cattle and beef cattle, managed by farmers who became business owners, constantly improving animal nutrition and breeding, and technifying management and facilities, to achieve higher and more efficient production. Overall, the current cattle system is principally intensive and specialized.

In Spain, the dairy and beef sectors represent 16 and 17%, respectively, of final livestock production, only behind pig production, which represents 39% (MAPAMA, 2018). With regard to cow milk production, Spain is the 8th most productive country in the EU-28, with 7,117 thousand tons (4.6% of total production), behind Germany, France, UK, Netherlands, Poland, Italy and Ireland (Eurostat, 2018). As for beef meat production, Spain is the 5th most productive country in the EU-28 with 667 thousand tons (8.4% of total production) behind France, Germany, UK and Italy (Eurostat, 2018).

The number of cattle farms in Spain is around 110 thousand, the majority of which are focused on beef meat production (MAPAMA, 2018). Fewer farms are devoted to milk cow production, the figure being around 15 thousand farms (MAPAMA, 2018). Overall, the total Spanish census of productive cows (older than 24 months) was 3.1 million animals, with 27% of females being involved in milk production and 63% in beef cattle breeding (MAPAMA, 2018).

The cattle industry has been adapting to demands in terms of food security, hygiene and quality, and society concerns in recent decades. The industry is also facing new challenges related to biosecurity, resistance to antimicrobials, climate change and sustainability, new consumer habits, and animal welfare. Researchers, companies and governments have been trying to find solutions, considering the profitability of each strategy implemented to ensure the success of this industry.

1.2. Animal Welfare

The animal welfare concept was born in 1964 with Ruth Harrison's book "Animal Machines", which described intensive conditions for farmed animals. The book impacted profoundly on world agriculture, public opinion and the quality of life of millions of farmed animals. In response to this book, moral and ethical concerns about animal welfare emerged in society, and the UK government set up "The Brambell Committee" to establish the scientific basis of animal welfare. Since then, the animal welfare concept has been widely studied and new approaches are investigated as social opinion changes.

1.2.1. Social and ethical approach of animal welfare

The importance attributed to different aspects of animal welfare varies between countries, cultures and individuals. As public opinion has great importance in animal welfare research, it is important to look briefly at the current situation.

The main findings of Eurobarometer (2016) survey illustrate, with high levels of agreement, that animal welfare is an important issue for Europeans. Europeans are divided on what they understand animal welfare to mean: 46% see it as "the duty to respect all animals", whereas 40% define it as "the way farmed animals are treated, providing them with a better-quality life". In the case of Spain, 51% prefer the first definition, while 24% prefer the second. Almost all Europeans consider the welfare of farmed animals to be important (94%) and that their welfare should be better protected than it is now (82%). A growing number of EU citizens since the last survey would like to have more information about the conditions under which farmed animals are treated in their respective countries (64%). To conclude, more than 90% of Europeans who think the EU should do more for animal welfare awareness, or think there should be EU laws obliging people using animals for commercial purposes to care for them, are more likely to agree with each of the statements than those with the opposing view. However, more than a third of EU citizens (35%) are not ready to pay more for products sourced from animal welfare-friendly production systems.

1.2.2. The concept of animal welfare

Most authors agree that three ethical concerns should be included when assessing animal welfare: the ability to live a natural life, feeling well (free from pain, fear and frustration, and experiencing positive emotions) and functioning well (healthy and within normal physiological and behavioral limits) (Fraser et al., 1997; Hewson, 2003). Various scientists have proposed restricted conceptions of animal welfare that relate to only one or other of these three concerns. In order to better enclose the three concerns, Fraser et al. (1997) conceptualized the animal welfare concerns expressed by recognizing three types of problems that may arise when the adaptations displayed by an animal do not fully correspond to the challenges posed by its current environment.

Problems were classified as follows:

- **Type 1:** animals possess adaptations that no longer serve a significant function in the new environment, then unpleasant subjective experiences may arise, yet these may not be accompanied by significant disruption to biological functioning.
- **Type 2:** the environment poses challenges for which the animal has no corresponding adaptation, then functional problems may arise, yet these may not be accompanied by significant effects on subjective feelings.
- **Type 3:** animals have adaptations corresponding to the kinds of environmental challenges they face, then problems may still arise if adaptations prove inadequate.

All three types of problems are causes of previous ethical concerns and they together define the subject matter of animal welfare science. Problems type 1 and 2 reflect natural-living concerns. Problems type 1 and 3 reflect concerns about the subjective experience of animals. Problems type 2 and 3 problems concerns about biological functioning.

Overall, Broom (1986) defined the concept of animal welfare as the animal's state as regards its attempts to cope with its environment. Poor welfare is classified in two ways: the first demonstrating that an individual has failed to cope with an environment, and the other indicating the effort (i.e. time and energy) involved in an individual's attempts at coping (Broom, 1986).

1.2.3. Assessment of animal welfare

Research on animal welfare requires parameters and tools to analyze it, that is to say welfare indicators linked to their assessment measures. When using such welfare indicators, it is the welfare of each individual rather than that of the group which must be assessed because social factors may be improving or exacerbating welfare of each individual (Broom, 1986). A wide range of welfare indicators should be used (Broom, 1986). A single indicator cannot show that welfare is poor because individuals vary in the methods which they use to cope with challenging conditions (Duncan and Filshie, 1979). Most of the research focused on negative indicators for detection of poor welfare, whereas little has been done about positive states of well-being. More research is needed on positive indicators and assessment measures of good welfare because even though disease, pain, low growth rates, impaired immunity, high glucocorticoid levels, etc. can define poor welfare, the absence of these symptoms cannot be considered indicative of good welfare.

A good indicator must meet three requirements: validity, feasibility and reliability (Knierim and Winckler, 2009). The first requirement, validity, means the indicator has a scientific well-documented association with animal welfare. The second requirement, feasibility, means the indicator can be recorded without too much extra work or cost. The third requirement, reliability, is that it must be possible to measure the indicator objectively with good robustness, referring to intra- and inter-observer variation or variation due to external factors, and accuracy, referring to the measuring being close to the true state of the indicator in question (i.e. high sensitivity and specificity).

Indicators are classified in two types:

- **Direct:** provide information of how the animal reacts to its environment, using animal-based measures to directly assess the animal's state. Animal-based measures analyze behavior, physiology, health and/or performance of the animal.
- **Indirect:** provide information about how risky the environment, such as housing or management system, is for the animal, using resource-based measures to assess the environment in which the animal are kept, or management-based measures to assess the management processes used on the animal. Resource-based measures analyze the housing system, and management-based measures analyze the management system.

Direct and indirect indicators are closely associated because indirect indicators need animal-based measures to be validated and established, but direct indicators are preferred because animal-based measurements are more closely linked to the welfare of animals. However, the context of each farming system (i.e., environment, resources, and management practices) can point to the causes of impaired welfare assessed by animal-based measures. Thus, it seems that resource-based and management-based measures have some advantages in fortifying overall welfare assessment, easing on-farm assessment without disturbing animals, or substituting animal-based measures with poor feasibility.

Given the wide spectrum of welfare indicators, it is necessary to look at the goals of each welfare assessment model because different combinations of indicators are required in each case. Welfare assessment models can be categorized in research, legislative requirements, certification systems, or advisory/management tools. These models may have various goals, such as welfare quantification, welfare assurance provision or/and welfare management. One important welfare assessment model is The European Welfare Quality® project, which developed an assessment protocol for cattle with scientific and standardized measures for analyzing on-farm welfare and qualifying each farm with a category, which makes it possible to recommend improvements to the farmers related to proper management of the farm (Welfare Quality Project, 2009). Welfare Quality® protocol focuses on animal-based measures, in contrast with other welfare assessment models that focus on resource and management measures (Botreau et al., 2009).

1.3. Animal Welfare in the Cattle Industry

Regarding the intensification of the system that farm species have been subjected to in recent decades, including cattle, animals have been coping with new environments such as facilities, management procedures, feeding protocols and even weather conditions, which have rarely been established considering their welfare. In reference to the conceptualization of animal welfare by Fraser et al. (1997), current cattle welfare research is focused mainly on biological functioning, involving type 2 and 3 problems, which arise due to the lack or weak match of animal adaptations to new environments which they are exposed to. Researchers have studied alternative housing or management systems that solved these welfare problems, simultaneously maintaining or improving animal performance, which ultimately is the main goal of farms for their economic livelihood. This has led to significant progress in welfare and management in recent years, but there are still unknowns to be solved in the different stages of cattle production. The aim is to understand the interplay between performance, health, and behavior of cattle.

Two specific issues, upon which this doctoral thesis is focused, will now be explained. Each issue is related to one of the two cattle sectors, and describes a current and relevant problem in terms of feeding system (beef cattle) or housing (dairy cattle) that affects animal welfare.

1.3.1. Feeding system in beef cattle

The National Animal Health Monitoring System estimated that 71% of feed yards surveyed were affected by digestive problems, and the respondents only observed digestive-related issues in 4.2% of the cattle (USDA, 2011). Records show a low level of detected morbidity of digestive problems compared with high mortality (USDA, 2011), which are between 30 and 42% of total registered mortality in feedlots, being responsible for between 0.17 and 0.42% monthly animal deaths (Smith, 1998). Ruminal acidosis is one of most common digestive disorders, and its mortality is related to the acute or clinical form of the disease. However, the subacute or subclinical form of the disease, named Subacute Ruminal Acidosis (SARA), is important because its unclear symptoms frequently unnoticed by farmers cause a reduced animal performance, the economic impact of which could be greater than in acute acidosis (Britton and Stock, 1989). In a study comparing different diets, Stock et al. (1990) observed that beef cattle with SARA produced a negative yield of $9.4 per animal in contrast with healthy animals.

To explain SARA disorder, it is important to contextualize it in feedlot cattle system. In recent decades, the cattle industry in Spain has turned to an intensive productive system. This current productive approach forced farmers to adapt the feeding system because a forage-based diet can not cover animal requirements, due to the limitation of rumen capacity. A common practice to meet the high nutritional requirements is to feed high-concentrate diets to beef cattle, by including large quantities of non-fibrous carbohydrates, mainly cereals, which add more energy in less volume than forages. In Spain, feedlot is the typical fattening cattle system, based on ad-libitum diets with 85-90% of concentrate and 10-15% of forage separately supplied (Bacha, 2002). The main advantage of this system is the high animal growth rate, which allows a short productive cycle (8-10 months). One disadvantage, on the other hand, is that the high intake of concentrate triggers a metabolic pathway, which increases the risk of SARA.

1.3.1.1. Subacute ruminal acidosis (SARA)

Ruminant feeding strategy is based on a symbiosis between the animal and ruminal microorganisms. The animal eats feed and promotes the appropriate ruminal conditions, allowing ruminal microorganisms to make forages digestible and produce short-chain fatty acids (SCFA) and microbial protein through the fermentation of feed. When changes or presence of undesirable substances in the diet disturb ruminal symbiosis, ruminal microbial population becomes unbalanced, causing digestive problems.

Subacute ruminal acidosis is a consequence of repeated periods of moderately low ruminal pH, which is unable to display the clinical symptomatology of ruminal acidosis. Although there is still no consensus on the definition of SARA, it is generally agreed that SARA occurs when the ruminal pH is lower than 5.5–5.8 for several hours a day (Plaizier et al., 2008; Zebeli et al., 2008), suggesting that duration below a certain pH threshold is more important than a low pH value as a single event anytime during the day.

This disorder is characterized by a decline in ruminal pH when SCFA production surpasses the absorptive, buffering and outflow capacity of the rumen (Aschenbach et al., 2011). In line with the current cattle system, the high intake of concentrate provides rapidly fermentable carbohydrates, in particular starch, which cause the accumulation of SCFA in the rumen and increase the acid load, and consequently, reduce ruminal pH. Despite this relationship, ruminal pH variation is more affected by ruminal digestible starch (97% of the variation) than by ruminal SCFA (32% of the variation) (Sauvant et al., 1999). Besides SCFA ruminal accumulation, an insufficient contribution of tampon substances to the rumen, and a poor rumen adaptation to high fermentable diets also contributes to reduce ruminal pH (Oetzel, 2003). The acid ruminal environment of SARA changes the rumen's microbial fermentation, further aggravating ruminal pH decline. However, Calsamiglia et al. (2008) reported that diet type also affects rumen microbial fermentation independently of the ruminal pH, focusing on the SARA approach to control ruminal pH as well as fermentation profile determined by the diet substrate.

Furthermore, high-concentrate diets reduce forage intake, which aggravate rumen conditions. This is explained by the ability of forage to stimulate chewing, which has been investigated extensively because of the relationship between chewing and the flow of salivary buffers into the rumen, which are required to neutralize fermentation acids and maintain desirable ruminal pH (Emery et al., 1960; Batajoo and Shaver, 1994;

Allen, 1997). However, high-concentrate diets increase the risk of SARA rather than being a cause of it, as suggested the wide individual variation in ruminal pH reported by some authors. Brown et al. (2000) used a model to induce SARA in beef cattle by switching animals in one day from a 50:50 forage to concentrate ratio diet to a concentrate-only diet, resulting in only 1 of 5 animals suffering SARA. Blanch (2009) induced ruminal acidosis in heifers by progressively increasing the level of concentrate over a 5-d period in a 100% forage diet which had been offered during the previous 3 months. Despite the abrupt change, 1 of the 6 animals did not suffer acidosis. This individual variation suggested that nutritional and physiological factors are not alone in causing SARA. Sauvant et al. (1999) suggested that changes in ruminal pH can be better explained by changes in intake speed rather than changes in acid load in the rumen. Thus, feeding behavior impact on the risk of SARA is altered by feed management and composition, farm management procedures and social hierarchy (González et al., 2009; Moya et al., 2015).

Diagnosis of SARA remains difficult because continuous ruminal pH measurement is the most reliable tool for research studies but costly in on-farm assessment, whereas indirect markers for a decline in ruminal pH (e.g. chewing and feeding activities) are more practical on-farm but with poor reliability (Humer et al., 2018). Therefore, preventive feeding strategies are more useful to control SARA in a herd.

1.3.1.1.1. Feeding strategies in subacute ruminal acidosis (SARA) prevention

A key challenge in management of SARA is to detect and treat the disorder in a timely manner. Focusing on the prevention of digestive-related diseases is paramount to reducing SARA prevalence. Ruminant animals possess complex and multi-faceted systems for maintaining stable ruminal pH, thus the nutritional approach to SARA prevention is also complicated. Because of the wide diversity of factors that can affect development of SARA, a wide range of feeding strategies can be used for the approach of this digestive disorder. As decreases in dry matter intake (DMI) have been reported (Hales et al., 2014; Swanson et al., 2017) with a level of forage inclusion greater than the typical range of 8 to 10% of forage used in feedlot finishing diets (Samuelson et al., 2016), reducing concentrate in the diet is not a good strategy to maintain animal performance.

Diet adaptation

Adaptation to high-grain diets is characterized by significant changes in the ruminal environment and ruminal bacterial population (Tajima et al., 2000). The rapid adaptation of cattle to high-grain diets is desirable because average daily gain (ADG) and gain efficiency are typically enhanced when high-concentrate diets are consumed, but this abrupt change makes the microbiota unbalanced, which leads to a non-physiological accumulation of SCFA and lactate in the rumen, resulting in lower ruminal pH (Goad et al., 1998; Coe et al., 1999), as well as decreased feed intake and performance (Koers et al., 1976; Owens et al., 1998). Some authors have reported that cattle adapted rapidly to diets of to 90% in less than 4 days have more variable ruminal pH response, lower intakes, and increased incidence of acidosis (Bevans et al., 2005; Brown et al., 2006). Bevans et al. (2005) reported greater variance in most pH values for rapidly adapted than for gradually adapted heifers, representing a greater opportunity for acidosis to occur in some individuals. Although data suggest that most cattle can be rapidly adapted to high-grain diets in a few incremental steps, minimizing acidosis in the most susceptible individuals requires decreasing the pace of grain adaptation for the entire group. Thus, feeding programs have been implemented to adapt feedlot cattle from a high-forage diet to a high-concentrate diet by gradually increasing the concentration of grain in the diet, starting with a diet of 45 to 55% concentrate and moving to an 85 to 95% concentrate diet, over a period of 3 or more weeks in order for both the animal and the rumen microbial population to have ample time to adapt to diets containing readily fermentable carbohydrates (Bevans et al., 2005; Brown et al., 2006). Although these protocols are generally successful at controlling acidosis, they do not eliminate it (Burrin and Britton, 1986).

Feeding method

Ruminants require forage in their diets to maximize production and to maintain health by sustaining a stable environment in the rumen (Allen, 1997). In a free choice feeding method, cattle consume 20% of their DMI as forage (Forbes and Provenza, 2000). However, the incidence of SARA is high when diets are offered as free choice (Østergaard and Gröhn, 2000) because this feeding method facilitates dietary preference, resulting in cattle selecting a higher proportion of concentrate and a lower proportion of forage, close to 10% in young cattle (Devant et al., 2000; Maekawa et al., 2002; González et al., 2008a; Faleiro et al., 2011). On the other hand, total mixed ratio (TMR) is designed to be a homogeneous mixture to make it difficult for animals to separate components, minimizing the selective consumption of individual components (Coppock et al., 1981). DeVries and von Keyserlingk (2009) reported that providing feed components as a TMR reduced the amount of sorting (against long forage particles and for short grain concentrate particles) in young dairy heifers. Consequently, TMR permited greater control of the forage to concentrate ratio consumed (Maekawa et al., 2002) and increased the amount of forage consumed, resulting in an increase in the time spent ruminating (Iraira et al., 2012). However, animals can still sort in TMR with a low proportion of forage, as has been demonstrated in dairy cows exhibiting more sorting for short particles and against long particles (Leonardi and Armentano, 2003; DeVries et al., 2007). Also, sorting behavior has been detected in growing calves (Miller-Cushon et al., 2013; Groen et al., 2015; Gordon and DeVries, 2016), growing heifers (DeVries et al., 2014; Madruga et al., 2017), and fattening heifers (Madruga et al., 2018) fed high-concentrate diets. Keunen et al. (2002), however, stated that dairy cows induced with SARA and feeding TMR increased their dietary preference for a feed of longer particle size. In this way and conversely to TMR, Moya et al. (2011) concluded that cattle with free choice diets can effectively self-select diets without increasing the risk of SARA and still maintain similar levels of growth and feed efficiency compared with TMR.

Feed bunk management

Bunk management practices that cause cows to eat fewer and larger meals more quickly may be associated with an increased incidence of SARA (Krause and Oetzel, 2006). Factors that can cause altered feeding patterns include infrequent feeding delivery, inconsistent feeding schedule, and limited bunk space and bunk competition.

A common and firmly held belief in the feedlot industry is that consuming feed at a constant rate with low daily fluctuations will lead to greater performance and decreased incidence of SARA (Galyean et al., 1992). Schwartzkopf-Genswein et al. (2004) conducted a study feeding finishing cattle with either a constant level or amounts of feed fluctuating by 10% above and below, concluding that inconsistent delivery of feed lowered ruminal pH, suggesting increased risk of SARA without impairing performance.

Another study conducted an experimental simulation where the time of feed delivery was delayed for some animals in a group of heifers in full view of each other. González et al. (2009) stated that the stress response of heifers fed with delays, as reflected in greater salivary cortisol, may lead to reduced concentrate intake, increased straw intake, and decreased size of the first meal after feed delivery, all of which are indicative of reduced appetite. Risk of ruminal acidosis did not increase because changes in feeding behavior were toward greater ruminal pH, but welfare and performance were compromised.

Feeding ruminants more than once daily results in a more constant ruminal pH and in lower postprandial ruminal pH decrease by minimizing starch intake per meal (Kaufmann, 1976). In dairy cows, Sutton et al. (1986) and Yang and Varga (1989) reported that increasing the feeding frequency of concentrate increases minimum ruminal pH but decreases average ruminal pH, concluding that increasing feeding frequency in ruminants might decrease the risk of acidosis, achieving more stable ruminal conditions. In heifers fed a high concentrate diet, Soto-Navarro et al. (2000) found that there was a tendency for pH to be lower in steers fed once daily compared with those fed twice daily, and Robles et al. (2007) demonstrated that feeding once daily did not cause ruminal acidosis but feeding twice daily reduced the range of pH values. Feeding behavior could explain this differential pattern because, although heifers spent similar times on chewing activities under any feeding frequencies, more stable ruminal conditions were probably achieved feeding twice daily due to the rumination pattern (Robles et al., 2007). Thus, Robles et al. (2007) concluded that increasing feeding frequency did not result in any significant advantage to avoid SARA but could be a practical way to better control the daily pH fall, without impairing DMI.

Social hierarchy in beef cattle is characterized by a dominance based on the availability of resources (e.g. feed), affected by an animal's motivation to gain access to feed and limiting subordinate animals (Val-Laillet et al., 2008). Thus, competition increases the variability in intake among individuals who use different coping mechanisms (Zobel et al., 2011; González et al., 2008b) which may increase ruminal acidosis (Cook et al., 2004; Nagaraja and Chengappa, 1998). González et al. (2012) reported that increased competition in feedlot heifers resulted in a reduction in daily bunk attendance, while feed intake increased at a rate 75% faster to achieve ad libitum consumption, which may reduce chewing. Accordingly, sufficient availability of feeding space may provide uniform opportunities of access to feed among heifers. González et al. (2008b) assessed the effects of 2, 4 or 8 heifers per feeder. More feeding space did not impair performance but may have improved group homogeneity of body weight (BW). Eating rate increased while time spent eating decreased as competition increased. Thus, heifers adapted to increased competition by changing their behavior, these forced changes being detrimental for animal welfare. The average ruminal pH was not affected, but that proportion of heifers with ruminal pH below 5.6 tended to increase linearly as competition increased, and ruminal lactate and blood haptoglobin indicated that the risk of SARA might increase with competition. González et al. (2008b) stated that four heifers per concentrate feeding place seems a reasonable upper limit considering performance parameters, but not with regard to SARA.

Type of grain and processing

Total ruminal organic acid production is less with grains containing less starch and that are less processed. So, the total quantity and rate at which starch molecules are converted to ruminal SCFA, lactate and gas are determined by the interaction of starch content, the grain processing method, and degree of processing. These factors can determine the risk of SARA. He et al. (2015) found that the substitution of wheat- for barley-grain in a finishing feedlot diet tended to increase the time that ruminal pH was below 5.8 and to change feeding behavior, tending towards shorter and smaller meals, something which has been previously suggested as a mechanism that feedlot cattle use to avoid the potentially harmful effects of highly fermentable diets (González et al. 2012). Factors such as the greater starch and lower fiber content, and nutrient digestibility of a wheat-based diet are associated with increased production of organic acids, with proportionately more propionate than acetate, and increased rumen osmolality, which can in turn inhibit feed intake, salivation, and the onset of rumination following meals (Oba and Allen, 2003). In a similar study by Moya et al. (2015), steers fed a wheat-grain diet, compared to a barley-grain diet, had a lower DMI and altered feeding behavior, characterized by lowered time and frequency of bunk attendance, without effects on growth performance. However, a greater concentration of cortisol in the hair is indicative of chronic stress and reduced animal welfare. Regarding grain processing, extensive processing techniques, such as steam flaking or high moisture corn, maximize grain degradability by providing easy access to its starchy core for both microbes in the rumen and digestive enzymes in the lower digestive tract. However, dry-rolling of corn results in lower animal productivity and efficiency than products such as steam-flaked and high-moisture corn (Owens et al. 1997). A balance is sought between animal productivity and ruminal health because a slow rate of fermentation is better for prevention of acidosis, but a faster rate of degradation is better for energetic efficiency (Owens et al., 1998).

Feed additives

Antibiotics, and particularly ionophores such as monensin, are the primary feed additives used in the feedlot industry to enhance the efficiency of ruminal fermentation. Different antibiotics, however, target different bacterial populations and therefore cause a specific shift of rumen microbial profile, resulting in different effects on feeding behavior and rumen pH. Researchers have shown that monensin decreases meal size and daily feed intake variation while increasing meal frequency (Stock et al., 1995; Erickson et al., 2003). As a result of the changes in meals and bacterial population, monensin can increase ruminal pH and moderate changes in pH (Erickson et al., 2003). However, EU legislation banned the use of antibiotics in animal feeds in January 2006 (OJEU, 2003). Most other feed additives and ingredients that have been researched have had no or inconclusive effects. For example, the addition of sodium bicarbonate to feedlot diets has had mixed results (Zinn, 1991; González et al., 2008c). Other feed additives, such as yeast-based products (Saccharomyces cerevisiae), have also been suggested as a useful tool to stabilize ruminal fermentation by providing metabolites (i.e., B vitamins, amino acids, organic acids) that work as stimulatory nutrients to specific fiber-digesting and lactate-utilizing bacteria (Moya et al., 2009; Fandiño et al., 2020).

Source of forage

Although most feedlot diets are primarily composed of grain, numerous aspects of dietary forage play a critical role in ruminal and animal health, including minimizing the risk of SARA. Forage sources used in most feedlot diets have different chemical and physical profiles, which makes determining forage value and equivalency difficult (Galyean and Defoor, 2003). The most common system of categorizing forage chemical characteristics is the neutral detergent fiber (NDF) method, which was devised by Van Soest (1967). Only NDF measures total fiber, and Galyean and Defoor (2003) concluded that NDF supplied by forage might be a useful method for exchanging forage sources in finishing diets (Galyean and Defoor, 2003; Salinas-Chavira et al., 2013; Swanson et al., 2017). Mertens (1997) explained that, biologically, NDF have been related to intake, feed density, chewing activity, digestibility, rate of digestion, and depression of digestibility associated with high levels of intake. However, NDF does not measure the physical characteristics of fiber such as particle size and density. These physical characteristics can influence animal health, ruminal fermentation and utilization, and animal metabolism independently of the amount or composition of chemically measured NDF. Although forage NDF has been correlated to ruminal pH (Allen 1997; Galyean and Defoor, 2003), some situations (Grant et al., 1990) indicate that reduced forage particle size is the primary cause of borderline acidosis. For this reason, NDF is less effective in formulating rations when finely chopped forages or nonforage fiber sources are used, the physical characteristics of fiber being critical for the optimal function of the rumen.

Particle size of forage

Different indexes have been developed to define the effectiveness of fiber, which in turn help to clarify the animal response used to assess this effectiveness. Mertens (1997) defined the physically effective NDF (peNDF) related to the physical characteristics of fiber, such as particle size, that influence chewing activity and the biphasic nature of ruminal contents (floating mat of large particles on a pool of liquid and small particles). Because peNDF relates only to the physical properties of fiber, peNDF is a more restricted concept than effective fiber (eNDF), which is related to the sum total ability of a feed to replace forage in a ration so that the percentage of fat in milk produced by cows eating the ration is effectively maintained. Conceptually, peNDF is related to fibrosity characteristics, roughage value index, physical structure, and fibrosity index. However, peNDF is based on the two fundamental properties of feeds that affect chewing: NDF and particle size.

The peNDF of a forage is the product of its NDF concentration and its physical effectiveness factor (pef). Mertens (1997) developed a system to determine the pef of a feed based on its ability to stimulate chewing. A limitation to using chewing time to indicate the physical effectiveness of feeds is the need to rely on book values to adjust the values for individual feed samples. Thus, measuring physical effectiveness of feeds based on particle length have been developed. The pef values determined by sieving are based on the concept that long particles retained on sieves represent particles that require chewing. The penn state particle separator (PSPS; Figure 1.1) is one method of measuring particle length and determining the pef of a feed or TMR, which consists of three sieves (19-, 8- and 1.18-mm openings), and a collection pan (Kononoff et al. 2003a). The pef is determined as the proportion of the total sample dry matter (DM) content retained on each sieve. A 1.18 mm sieve size has been widely used as the size in which feed particles retained on or above are considered peNDF. It was determined that 1.18 mm was a threshold particle size for both cattle and sheep for greatly increased resistance to particles leaving the rumen, and < 5% of fecal particles are generally retained on a 1.18-mm sieve (Poppi et al., 1980). It should be noted that a wet-sieving technique was used in these studies to measure particle size and this procedure is very different from the dry vertical sieving procedure used by Mertens (1997) or PSPS to develop the pef of feeds (using particles retained on a 1.18-mm sieve). Maulfair et al. (2011) compared 4 TMR diet of dairy cows differing in particle size (as-fed %) and determined that rumen digesta particle size increased with increasing ration particle size for sieves ≥3.35 mm, and remained the same for sieves < 3.35 mm. Fecal particle size was not different among rations with more than 36% of particles being retained on an 1.18-mm sieve or larger. Maulfair et al. (2011) concluded that critical particle size for rumen escape is larger than 1.18 mm in dairy cattle, as suggested by Yang et al. (2001) and Oshita et al. (2004). On the basis of the literature, Heinrichs (2013) recommended 4 mm as a more suitable particle size for estimating peNDF, and introduced an additional 4-mm sieve in the PSPS sieving method.

Figure 1.1 Penn state particle separator.

Physically effective NDF is related to the formation of the ruminal mat, which may be a critical factor for selectively retaining fiber particles in the rumen, determining the dynamics of ruminal fermentation and passage, and stimulating rumination. It is also related to animal health because ruminal pH and the pattern of fermentation may both be a function of the production of salivary buffers during eating and rumination. A wide range of studies reported the relationship between peNDF and ruminal pH and feeding behavior such as chewing. As some studies only measured mean ruminal pH (instead of duration below critical SARA pH), which does not reflect the extent of variation in pH among cows or daily fluctuations, some authors did not report the effect of peNDF on ruminal pH (Kononoff et al., 2003b; Einarson et al., 2004; Beauchemin and Yang, 2005; Yang and Beauchemin, 2006a), although almost all of them found increased chewing activity. Despite this, Grant el al. (1990) demonstrated that feeding shorter particle size results in reduced chewing time and ruminal pH, and some authors had positively correlated the particle size and peNDF with chewing activity and ruminal pH (Yang and Beauchemin, 2006b; Tafaj et al., 2007; Zebeli et al., 2006; Yang and Beauchemin, 2009). However, Yang and Beauchemin (2009) concluded that the correlation of mean ruminal pH with chewing was lower than the correlation of mean ruminal pH with peNDF. Other research has shown that dairy cattle may be capable of selecting feeds that can stimulate chewing to attenuate the effects of low ruminal pH. Keunen et al. (2002) demonstrated that when SARA was induced in cows, they increased their preference for long alfalfa hay over pelleted alfalfa. DeVries et al. (2008) determined that cows generally increased their sorting for medium particles and against short and fine particles and exhibited no change in sorting long particles when challenged by SARA. Maulfair et al. (2013) showed that during a bout of SARA, cows were able to alter their diet preference for higher peNDF and slower starch fermentability.

The extent to which DMI is regulated by distension in the rumen depends upon the animal’s energy requirement and the filling effect of the diet offered (Allen, 2000). Increasing particle size of the diet lowers the passage rate of digesta and may decrease net fiber degradation in the rumen, due to a lower availability of surface area for microbial attack, and thus decreasing feed intake and nutrient uptake (Tafaj et al., 2007; Storm and Kristensen, 2010). However, a meta-analysis examining effects of particle size of TMR detected no effects on intake (Tafaj et al., 2007). But some studies said that increasing forage particle size had negative effects on DMI (Kononoff et al., 2003b; Einarson et al., 2004; Teimouri Yansari et al., 2004). Tafaj et al. (2001) observed that reduction of particle size in cows and sheep linearly increased DMI in low concentrate diets, but no effect on DMI occurred in high-concentrate diets probably because ruminal fill was not limiting DMI. There was no effect of forage particle size on DMI for diets containing about 0.40 of forage (Beauchemin et al., 2003; Beauchemin and Yang, 2005). However, dietary particle size is reported to affect DMI when diets contain about 0.60 forage (Schwab et al., 2002; Kononoff et al., 2003b). Although particle size seems to affect DMI, the amount of readily fermentable carbohydrates in the ration determines important metabolic changes in the rumen, which may affect DMI of dairy cows to a greater extent than forage particle size (Tafaj et al., 2007).

The impact of peNDF has been more thoroughly researched in dairy cattle than in beef cattle. Few studies have investigated the effect of particle size of the diet in beef cattle. Gentry et al. (2016) suggested that increasing particle size of forage may be a means to decrease forage inclusion while maintaining rumination and performance. Weiss et al. (2017) concluded that a diet with a lower inclusion of roughage with a larger particle size may stimulate rumination at the same level as one with a higher inclusion of roughage with a smaller particle size. Though not well defined, the ideal particle size of feed in finishing diets would promote intake, generate rumination, maintain desirable performance, and prevent acidotic events (Gentry et al., 2016). NRC (2000) suggested minimum 20% eNDF in high-concentrate diets of feedlot cattle to ensure ruminal pH above 6.2. Mertens (2002) recommended 15% peNDF (on DM basis) for feedlot cattle, with a range from 12 to 18%. Fox and Tedeschi (2002) suggested that beef cattle feedlot diets should have between 7 and 10% peNDF (on DM basis) to keep ruminal pH above 5.7. These recommendations were based on the equations of Pitt et al. (1996) and mentioned in NASEM (2016). Sarhan and Beauchemin (2015) concluded that empirical models that rely on only dietary peNDF intake to predict mean ruminal pH in beef cattle showed poor correlation between measured and predicted values ($R^2 < 0.52$), although the Pitt et al. (1996) model was one of the most reliable. Surprisingly, there is a lack of experimental research about peNDF requirements in beef cattle feedlot diets which seek to ascertain the optimum level that ensures a correct rumen function and reduces the risk of SARA, while maintaining proper performance and animal behavior.

1.3.2. Housing system in dairy cows

In dairy cow farms, the main problems are lameness, mastitis and reproductive problems (Stanković et al., 2014), which are health disorders usually caused by the environment where animals live, such as inappropriate floors, ineffective foot trimming and milking routine or poor nutrition. One important environmental factor is the housing system used in the farm, where cows are kept for part or all of the day. The housing system can affect dairy cow welfare and performance and has a major influence on the ecological footprint and consumer perception of dairy farming. Although tie stall barns remain popular in some countries, the most widespread solutions for housing dairy cattle are straw yards and freestall barns. Straw yards, which are more used in beef cattle than in dairy cows, are usually used in nursing and parturition pens. In recent decades, freestall has become established as the standard housing solution for dairy cows (Bewley et al., 2017). However, in recent years, research has demonstrated that freestall housing may compromise animal welfare, reducing animal comfort and foot and leg health, and produce large amounts of liquid manure, which is known to contribute to emission of greenhouse gases (Petersen, 2018). Moreover, consumer concerns about the conditions of dairy cows in intensive systems and the development of their natural behavior have fostered interest in alternative housing solutions. Compost-bedded pack (CBP) barns are a relatively new housing system that, compared with freestall, appears to improve cow comfort and better foot and leg health, and minimize the risks in udder health associated with straw yards (Eckelkamp et al., 2014). Producers also emphasize improvements in manure storage and quality. In the next section, we focus on the CBP barn because it corresponds with the system studied in the present doctoral thesis.

1.3.2.1. Compost-bedded pack (CBP) barns

Compost-bedded pack has garnered strong global interest in the past decade. Cows are provided with an open bedded pack area for resting and exercise (Figure 1.2a), separated from a concrete feed alley by a high retaining wall (Janni et al., 2007; Klaas et al., 2010; Black et al., 2013). The entire pack is tilled daily (Figure 1.2b), mixing cow excreta into the bedding to promote pack composting, and the area per cow required is generally higher than that in other housing systems (Janni et al., 2007). Although all CBP worldwide share common characteristics like an open bedded area and periodical pack aeration, notable differences can be found among the systems developed in different countries and climates. American and Israeli CBP concepts are

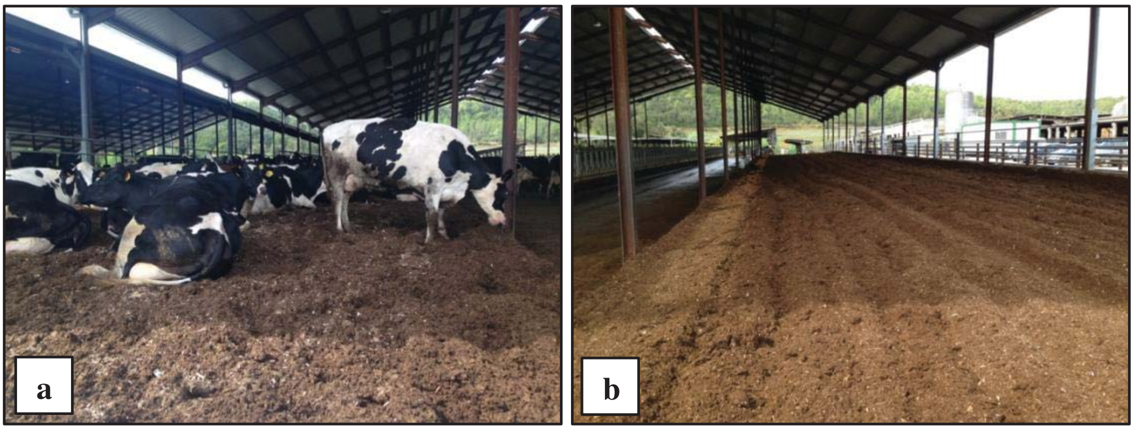


Figure 1.2 Compost-bedded pack (CBP) barn with dairy cows (a) and after tilling (b).

quite different, and appear to have provided a basis for the development of other systems worldwide. In short, US CBP is based on a smaller area per cow with frequent pack aeration and addition of bedding material, whereas Israeli CBP is based on large space allowances with less pack aeration and bedding material needed.

1.3.2.1.1. Impacts of compost-bedded pack (CBP)

According to Wagner (2002), CBP were first developed in Virginia during the 1980s with the aims of increasing cow comfort, improving longevity, and ease of farm chores and reduced building costs. However, all authors agree that the benefits reported in CBP system can only be achieved by adequate pack management resulting in proper pack performance (e.g. bad pack management increases pack moisture, which makes the pack too soft and cows sink into the pack, limiting cow comfort).

Cow comfort, behavior and reproductive performance

Endres and Barberg (2007) concluded that CBP can be an adequate housing system for dairy cows because their observations of lying behavior, social interactions, and natural lying positions were not substantially different from those reported in the literature for other types of housing. Later, some authors found improved lying behavior in CBP compared with freestall. Eckelkamp et al. (2014) showed that cows after transitioning from freestall to CBP spent more time lying down (9.6 vs. 13.1 h/d) and had more lying bouts (17.3 vs. 26.7 bouts/d). Borchers (2018) observed longer lying times in CBP (738.2 min) than in freestall (606.8 min). Fernández et al. (2020) observed longer time lying in the resting area in CBP than in freestall (96.5 vs. 56.4 % over 6h/d) and similar times needed to lie down (4.9 s). Ouweltjes and Smolders (2014) measured higher duration of lying down movement (6.3 s) in freestall than in CBP (4.8 s). Also, Ouweltjes and Smolders (2014) compared CBP with straw yards and Fernández et al. (2020) compared CBP with bedded pack with sawdust, both reporting that cows in CBP lay down more slowly than those in other housing systems. This result might have been caused by the daily tilling of CBP, which made it very soft and cows had to pull their legs out from the bedding before lying down, thus increasing the time required to lie down by approximately 1 s (Ouweltjes and Smolders, 2014). This finding indicates that the bedded pack should be soft to provide a comfortable and healthy surface but should also have adequate load-bearing capacity.

In addition, CBP are sometimes perceived to provide more natural living conditions for housed animals (Endres and Barberg, 2007). This is because the open pack area and the soft surface on which cows can stand, walk, and rest in CBP is more like the pasture environment, compared with freestall systems. These more natural living conditions decrease behavioral limitations, which may result from individual stalls and concrete paving, and allow the expression of natural cattle behavior. Moreover, because heat detection is primarily based on behavior monitoring, CBP has the potential to increase the percentage of heats observed (Black et al., 2013) and consequently improve reproductive performance, increasing average pregnancy rate (Barberg et al., 2007b) and reducing calving interval, number of days to first service, and days open (Black et al., 2013).

Cow longevity

Improved longevity is one of the most common reasons reported by producers for adopting the CBP system (Janni et al., 2007; Barberg et al., 2007a). However, several studies have evaluated the effects of CBP on culling rates and herd turnover rates, and the results are not completely consistent (Fulwider et al., 2007; Lobeck et al., 2011). Differences in culling rates may be explained by the complexity of factors affecting culling rates in different farm scenarios (Leso et al., 2020). In addition, some of the inconsistency may have arisen from poor management of the bedded pack. However, further and more detailed investigation of longevity in CBP is recommended.

Manure quality and storage

In CBP, feces and urine produced by cows over the bedded area are absorbed or mixed in the pack and can be handled as solid manure. This aspect is often perceived as an advantage and is one of the reasons for producers to build CBP (Leso et al., 2013). The pack area in CBP provides bedding storage for long periods, between 6 and 12 months (Barberg et al., 2007a; Galama et al., 2014). Solid manure can be used for direct land application, stored for future use, or further composted to improve manure quality and stability (Bewley et al., 2017). The amount of solid manure produced in CBP depends on several factors, including the amount and type of bedding material used, area per cow, and composting process. In CBP with a scraped (or slatted) feed alley, liquid manure is also produced, which must be handled in specific facilities.

Milk production

Some authors indicate that milk production increases after moving to CBP (Barberg et al., 2007b; Black et al., 2013). Greater milk production than in other housing systems such as freestall might be expected because CBP has the potential to improve cow comfort. Also, the authors acknowledged that, beyond the housing system, changes in management probably occurred in the process of moving to the new CBP that could potentially have contributed to the observed increase in milk production (Barberg et al., 2007b; Black et al., 2013). Although, a comparison of milk yield of cows housed in CBP and freestall did not show a clear difference (Lobeck et al., 2011; Costa et al., 2018), high levels of milk production are possible in CBP.

Lameness and hock lesions

Compost-bedded pack barns are thought to be healthier for cows than freestall housing systems due to the lower exposure to concrete surfaces and injury-causing obstacles (Bewley et al., 2017). The literature suggests that CBP, compared with freestall, involves improvements in foot and leg health (Fulwider et al., 2007; Lobeck et al., 2011; Burgstaller et al., 2016; Costa et al., 2018). Producers with CBP have also indicated that they were able to keep lame cows in the herd longer because the cows could stand up and lie down on the bedded area more easily (Barberg et al., 2007b). Eckelkamp et al. (2014) demonstrated that lame cows showed shorter lying times than sound cows in freestall system, but after transitioning to CBP, lame cows had similar lying times to sound cows. However, the reported results are not completely consistent, and large variations have been reported in the prevalence of both lameness (Lobeck et al., 2011; Black et al., 2013; Eckelkamp et al., 2016a; Burgstaller et al., 2016; Costa et al., 2018; Fernández et. al, 2020) and hock lesions (Shane et al., 2010; Klaas et al., 2010; Lobeck et al., 2011). Shane et al. (2010) suggested that the prevalence of hock lesions and potential lameness in CBP can be affected by the type of bedding material, probably due to the CBP performance of each material.

Cow hygiene

Studies regarding the hygiene of cows in CBP have shown inconsistent results, presenting wide variation in cows' hygiene scores (Shane et al., 2010; Lobeck et al., 2011; Black et al., 2013; Fernández et. al, 2020) and dirty cow prevalence (Klaas et al., 2010; Black et al., 2013; Costa et al., 2018). Compared with freestall, cows housed in CBP have comparable or poorer hygiene levels (Lobeck et al., 2011; Ofner-Schröck et al., 2015; Eckelkamp et al., 2016a; Fernández et. al, 2020). Hygiene of cows in CBP strongly depends on the CBP management and performance of the pack (Black et al., 2013; Fávero et al., 2015; Eckelkamp et al., 2016b). CBP moisture is the most important parameter because wet materials adhere more easily to animals, so increased pack moisture results in poor hygiene scores (Black et al., 2013; Eckelkamp et al., 2016a). CBP temperature and weather conditions also affect cows' hygiene, being cleaner animals associated with high CBP temperatures because they help to evaporate CBP moisture (Klaas et al., 2010; Black et al., 2013). In temperate climates, cows in CBP tend to be dirtier during winter because adequately maintaining dry bedding in cold and humid weather can be challenging (Lobeck et al., 2011; Black et al., 2013; Eckelkamp et al., 2016b). Ofner-Schröck et al. (2015) also emphasized the importance of the stocking density in this issue.

Udder health and microbiological population of the pack

Udder health traits reported in the literature suggest that adequate udder health can be maintained in CBP (Barberg et al., 2007b; Lobeck et al., 2011; Eckelkamp et al., 2016b). However, CBP environment appears to be risky to udder health because pack temperatures measured in most CBP indicate that the pack is biologically active but without high enough temperatures for pathogen devitalization and bedding sanitization. Thus, microbiological analyses indicate that most mastitis-causing bacteria thrive at the temperatures recorded in the composting pack studied (Black et al., 2014; Fávero et al., 2015). Concern about the risk of mastitis in CBP is explained because bedding bacterial counts in CBP are high (ranging from 7.0 to 8.9 $\log^{10}$ cfu/g), and bacterial counts and type of bacteria in the bedding material are positively correlated with bacterial counts on the teat ends (Hogan and Smith, 1997; Zdanowicz et al., 2004), and the rates of clinical mastitis in lactating dairy cows (Hogan et al., 1989). Most authors have highlighted the importance of applying correct pack management procedures to keep the layer surface dry to achieve acceptable cow hygiene and a lower risk of mastitis. Also, excellent teat preparation procedures in milking have been recommended for dairies with CBP (Janni et al., 2007; Lobeck et al., 2012; Black et al., 2014).

1.3.2.1.2. Management and performance of the pack

Improvements with CBP greatly depend on pack management that should aim at providing a hygienic and comfortable surface for the cows. The most important characteristic of the pack is its moisture content. High pack moisture is associated with increased prevalence of dirty cows, higher mastitis risk, reduced cow comfort, and higher gaseous emissions. The optimal moisture level for CBP ranges between 40% and 60-65% (Janni et al., 2007; Black et al., 2013). To keep the moisture level in the optimal range, water produced by the animals through excreta must be evaporated and/or absorbed. Absorbing water in excess requires the addition of dry bedding material. The periodic addition of dry bedding must be done at the correct time because any delay may resulting in rapid deterioration of pack conditions. But to reduce the amount of bedding needed, evaporation must be promoted by maintaining an active composting process which increases the temperature of the pack. For effective composting, recommended CBP temperature at pack depths of 15-31 cm ranges from 43.3 to 65.0°C (NRAES-54, 1992; Janni et al., 2007; Bewley et al., 2013). The active composting process is achieved by frequent and consistent pack aeration. When walking and lying down, the animals compact the bed surface, thus reducing the porosity of the material and consequently decreasing the amount of oxygen available for the composting process and the pack surface exposed for drying. Aeration restores pack porosity, thereby enhancing the composting process and drying rate (Janni et al., 2007; Damasceno, 2012), and allows for incorporation of fresh manure into the top layer of the pack, thus providing a cleaner lying surface for the cows (Shane et al., 2010). Twice daily tilling of the pack is recommended, even though some producers choose to till 1 to 3 times per day depending on CBP performance (Barberg et al., 2007a; Black et al., 2013; Black et al., 2014). Starting a new pack with at least 50-cm depth may allow an adequate composting process to be achieved at an early stage, and the pack should be tilled at a depth of 25 to 30 cm (Janni et al., 2007). Increasing tilling frequency and depth leads to higher pack temperature (Black et al., 2013). Stentiford (1996) indicated that higher compost temperatures tend to be achieved when the pack moisture is between 40 and 60%. As heat production within the pack is thought to improve evaporation, maintaining optimal pack chemical and physical characteristics in CBP is important to support rapid and consistent bacterial growth. Because aerobic processes produce more energy than anaerobic processes, high oxygen availability is crucial for optimal composting (FAO, 2003). Studies on the composting process have indicated that faster organic matter (OM) degradation occurs when the C:N ratio is in the range of 25:1 to 30:1 and the pH remains below 8 (FAO, 2003). As dairy cow feces have a low C:N ratio, ranging from 15:1 to 19:1 (Rynk et al., 1992; Leonard, 2001) and most commonly used bedding materials are dry and have a very high C:N ratio, the addition of dry bedding is also done to keep the pack C:N ratio within the optimal range. Galama et al. (2014) suggested that the barn should be cleaned out and a new pack should be started when the pack decreases to a C:N ratio of 15:1. Below this level, composting appears to be inhibited in CBP, and higher losses of nitrogen may occur.

1.3.2.1.3. Performance-related external pack parameters

Building orientation and the correct installation of enough fans can help to achieve proper barn ventilation which can improve pack drying rate. For this reason, a proper design of the CBP facilities in each context is crucial for the success of the pack. Apart from budget, other factors like climate, bedded pack area per cow and bedding material, may influence CBP design.

Climate

Environmental parameters are strictly associated with the pack drying rate (Eckelkamp et al., 2016b). Evaporation of the pack was found to be associated with ambient temperature, relative humidity, air velocity, sunlight exposure and pack surface temperature (Black et al., 2013). In temperate climates, maintaining dry bedding adequately during the winter may pose some challenges because cold and humid weather limits evaporation of water from the pack, and large amounts of dry bedding may be necessary to absorb excessive pack moisture. Increasing air temperature increases internal pack temperature and decreases pack moisture (Eckelkamp et al., 2016b). For this correlation, frequent aeration during winter may result in an excessive heat loss from the pack, thus disturbing the composting process.

Bedded pack area per cow

The bedded pack area per cow is one of the most important parameters in CBP design. The area per cow in different CBP systems can vary widely depending on several factors, but at least 7.4 m^2/cow for 540-kg cows should be provided (Janni et al., 2007). Because evaporation mainly occurs at the surface of the pack, increasing the area per cow in CBP generally results in drier bedding and reduced utilization of bedding materials, thereby decreasing running costs. However, a larger area per cow is also associated with higher initial barn costs. For this reason, the cost and availability of bedding as well as barn construction costs should be considered when designing CBP. A larger area per cow is recommended when the availability and cost of bedding materials are limiting or the construction cost is low, whereas decreasing the barn surface may be convenient in situations in which the bedding materials are inexpensive and readily available, or construction costs are high (Leso, 2015).

Bedding material

Sawdust, wood shavings and a mixture of them are the most common bedding materials used in CBP (Barberg et al., 2007a; Galama et al., 2011; Leso et al., 2013; Mota et al., 2017; Fernández et al., 2020). Other bedding materials have also worked: ground soybean straw (Janni et al., 2007); green or kiln-dried shavings with sawdust, or soy hulls with shavings and sawdust (Black et al., 2013); rice straw and coffee husks (Mota et al., 2017). Wood materials appear to be particularly suited to CBP, owing to their high energy content and high C:N ratio (Shane et al., 2010). Sawdust is appreciated for its high absorption capacity, whereas adding shavings or wood chips may be beneficial to maintain a loose structure in the pack. However, the use of fresh or wet sawdust reduces the water absorption capacity and may pose a risk of mastitis due to increased concentrations of *Klebsiella* spp. (Janni et al., 2007; Bewley et al., 2013). Shane et al. (2010) tested several different substrates, including pine sawdust, corn cobs, pine wood chip fines, and soybean straw, and found that almost any organic material can work in CBP if proper bedding management is applied on a consistent basis. The authors concluded that ideal bedding material for CBP should be dry (less than 25% initial moisture), be processed to less than 2.5 cm long, offer structural integrity, and have good water absorption and water-holding capacity. The implementation of these characteristics in the type of bedding material type enables a proper performance of the pack and the success of the CBP. Thus, an important source of variation in all aspects related to CBP appears to be associated with the type of material used as bedding.

Alternative materials used in CBP include cereal straw, corn stalks, coconut fiber, coarse hay, coffee husks, peanut shells, dried manure, and compost from organic waste (Bewley et al., 2017). Straw and corn stalks are not recommended for use in CBP due to difficulties in tilling with normal equipment and excessive pack compaction (Janni et al., 2007). Nevertheless, Galama et al. (2014) reported that cows housed in CBP bedded with straw have very low somatic cell count (SCC). Fine processing of corn cobs and any type of straw is strongly recommended for use in CBP (Shane et al., 2010). Such fine materials may be used in a mixture with sawdust or other wood materials. Some experience in CBP with inorganic bedding materials (e.g., sand and waste wallboard) has been found to be unsuitable for use in CBP because they do not promote composting (Galama et al., 2011).

Although the CBP housing system generally requires a greater amount of bedding material and has higher bedding costs than freestall (Black et al., 2013), the potential improvements in cow health, may offset these costs. Bedding utilization ranges between 8.2 and 25.6 m^3/cow per year depending on the bedding source and climate of the barn location (Janni et al., 2007; Black et al., 2013; Leso et al., 2013). The availability and cost of bedding materials are regarded as the main limits to the adoption of the CBP housing system (Barberg et al., 2007a; Leso et al., 2013). Wood-based materials, which are commonly used in CBP, are becoming increasingly popular because of market demand for renewable energy sources, and will probably become scarcer and more expensive in coming years. Thus, flexibility in bedding choice may play an important role in determining the economic sustainability of CBP systems. Further research should therefore focus on identifying alternative sources of bedding materials to be used in CBP. Inexpensive and widely available materials such as organic by-products or even waste materials (where allowed by the law) should be prioritized. When evaluating alternative materials, attention must be paid to microbiological safety of the pack.

1.4. Justification of the Topic

What the cattle industry issues addressed in this thesis have in common is that they are on-farm management processes affecting farm systems related to animal welfare. The purpose of the project was to use farm management strategies to improve both farm systems and animal welfare. According to Fraser et al. (1997), these suggested management strategies are tools to modify the environment to enhance animal adaptation, and thus achieve better animal welfare. In the project, problematic environments are related to feeding system in beef cattle and housing system in dairy cows. The suggested management strategies would turn the productive cattle conditions into a more natural living system in order to avoid biological functioning concerns that could impair animal health and performance.

In the first study, the current issue in cattle feedlots related to the feeding system is focused on the control of SARA through the physical composition of the diet. The current intensive system of fattening beef cattle based on high-concentrate diets increases the risk of SARA. This disorder disturbs ruminal balance reducing animal health and intake, thus impairing animal performance. Increasing forage intake in the diet reduces the risk of SARA because particle size and peNDF are increased, promoting chewing. Several studies have estimated a range of optimal peNDF in dairy cow diets, but there is less experimental research in beef cattle about this topic.

The farm management strategy to test in the context of fattening cattle is whether an optimal peNDF requirement in feedlot diets can be established to minimize the risk of SARA in beef cattle by testing increased proportions of peNDF in a high-concentrate diet fed to beef heifers (Figure 1.3). We hypothesized that increasing the proportion of peNDF in the diet should decrease the risk of SARA. As SARA and increasing peNDF proportion in the diet may affect intake, the first step was to compare intake among diets with different proportions of peNDF. This was assessed by animal-based measures linked to the direct indicator of performance. Secondly, as beef cattle usually perform sorting behavior in different conditions, we were interested to compare sorting behavior among diets, using animal-based measures linked to the direct indicator of sorting behavior. As increasing peNDF proportion in the diet increases ruminating and chewing, the third step was to compare animal behavior among diets,

Figure 1.3 Experimental diet for beef cattle composed by barley straw and concentrate, and with a specific proportion of physically effective fiber (peNDF).

which was also assessed by animal-based measures linked to the direct indicator of animal behavior. And finally, as SARA is characterized by ruminal pH lower than a pH threshold, and increasing peNDF proportion in the diet increases ruminal pH, we also decided to compare time under critical ruminal pH among diets, using animal-based measures linked to the direct indicator of physiology.

In the second study, the current issue in dairy cow farms related to the housing system is focused on the newest CBP barns. This new housing requires higher amounts of bedding material, so flexibility in bedding choice may play an important role in determining the economic sustainability of this system. A limited range of bedding materials are used in this increasingly popular housing system world-wide, wood-based materials, specifically sawdust, being the main bedding used in CBP. Research should focus on identifying alternative sources that perform three important things in this system: good composting, safe microbiological counts and acceptable animal welfare. A material which could meet these criteria is forest biomass, a byproduct resulting from forest cleaning that is mostly composed of tree bark and vegetal fibers. As accumulation of high fuel loads over large areas is the main cause of large fires (Minnich, 2001; Oliveras et al., 2005), it would be necessary to find new ways to reduce these high fuel loads, such as the use of forest biomass as bedding for livestock farming.

The management strategy to test in the context of dairy cows is whether forest biomass will work as bedding material in CBP barns. We hypothesized that forest biomass (Figure 1.4a) could work as well as sawdust (Figure 1.4b), the most commonly used bedding material. As a proper management of the pack is the basis of the success of the CBP system and its improvements, the first question to be answered was whether CBP with forest biomass as bedding material composted similarly to or better than CBP with sawdust as bedding material. This first question was assessed by resource-based measures linked to the indirect indicator of pack performance. As high microbial counts characterize the CBP system, second issue to address was whether CBP with forest biomass led to similar or lower microbial counts than CBP with sawdust. This second question was assessed by resource-based measures linked to the indirect indicator of pack microbial population. And finally, as potential improvements in cow health and comfort are fundamental in this housing system, we also wanted to know how works CBP with forest biomass compared to CBP with sawdust in terms of welfare. This third question was assessed by animal-based measures linked to direct indicators such as behavior and health.

Figure 1.4 Bedding materials for compost-bedded pack (CBP): forest biomass (a) and sawdust (b).

1.5. References

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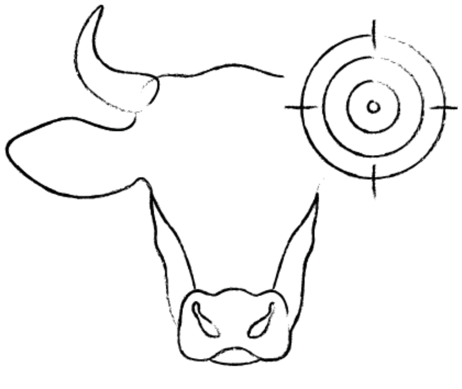


CHAPTER 2.

Objectives

The main objective of the current thesis was to test some farm management strategies in cattle intensive production systems to improve housing and feeding systems, as well as animal welfare. To accomplish this aim, two different cattle farm issues were studied. The first was the feeding system of beef cattle in relation to the physical composition of the diet and its effect on ruminal pH, while the second was the housing system of dairy cows in relation to the quality of the bedding material and the comfort of the animals. Both issues are current concerns in each productive stage of cattle intensive production systems.

The specific objectives were:

1. To establish the optimal level of physically effective neutral detergent fiber to minimize the risk of subacute ruminal acidosis in fattening beef cattle fed high-concentrate diets regarding intake, feeding and animal behavior, and rumen pH.

2. To compare the effect of forest biomass as an alternative substrate to sawdust, traditionally used in the resting area of compost-bedded pack barns of dairy cows, on pack performance (temperature, moisture and C:N ratio), pack microbial content and animal welfare (resting behavior and animal behavior).

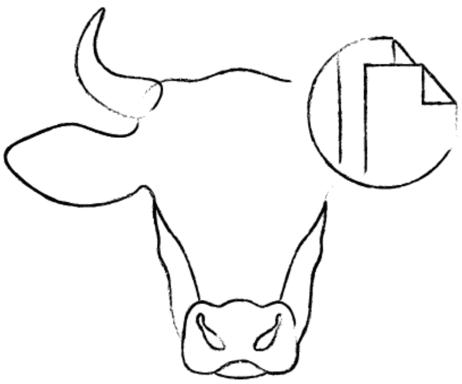
3. To ascertain the agronomic characteristics of the compost-bedded pack made with forest biomass or sawdust, and then the agronomic characteristics of both composted materials after conducting an additional composting process once the cows had been moved away.

To achieve these specific objectives, the following studies were conducted:

- **Study 1.** Increasing the content of physically effective fiber in high-concentrate diets fed to beef heifers affects intake, sorting behavior, time spent ruminating and rumen pH.

- **Study 2.** Effect of forest biomass as bedding material on compost-bedded pack performance, microbial content, and behavior of nonlactating dairy cows.

- **Study 3.** Agronomic characteristics of the compost-bedded pack made with forest biomass or sawdust.



CHAPTER 3.

Published Articles

3.1. Increasing the content of physically effective fiber in high-concentrate diets fed to beef heifers affects intake, sorting behavior, time spent ruminating and rumen pH

Published in Journal of Animal Science, 2020,
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L. Llonch, L. Castillejos, and A. Ferret.

Animal Nutrition and Welfare Service,
Dept. of Animal and Food Sciences,
Universitat Autònoma de Barcelona, 08193-Bellaterra, Spain

3.2. Effect of forest biomass as bedding material on compost-bedded pack performance, microbial content, and behavior of nonlactating dairy cows

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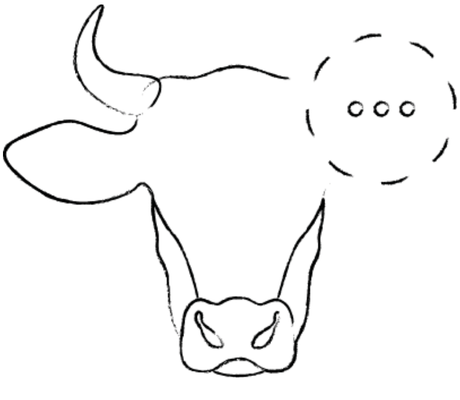
L. Llonch, L. Castillejos, E. Mainau, X. Manteca, and A. Ferret.

Animal Nutrition and Welfare Service,
Department of Animal and Food Science,
Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain



CHAPTER 4.

Supplementary Documentation

Agronomic characteristics of the compost-bedded pack made with forest biomass or sawdust

4.1. Abstract

To ascertain the agronomic value of the material resulting from the compost-bedded pack (CBP) used as a housing system for dairy farms, a cross-over experiment was designed with 8 dry non pregnant Holstein cows. The study was performed in 2 11-week periods with a 4-week washout period between them. Treatments were: 1) CBP with bedding material of sawdust (CBP-S) and 2) CBP with bedding material of forest biomass (CBP-FB). During the experiment samples were taken from the raw bedding materials (S and FB), and from CBP-S and CBP-FB at week 11. In addition, we conducted an additional study preparing 2 piles, one of each CBP material, to obtain a composted material after 3 months of the composting process, during which time samples were taken. With regard to the bedding materials, granulometry and bulk density together with the acidic pH and the C:N ratio of FB made it a suitable bedding material to be used as CBP. However, the greater moisture recorded in FB than in S can limit its ability to absorb liquid manure. The degree of stability of the organic matter after 11 week was greater in CBP-FB than in CBP-S, in agreement with the differences recorded in the raw bedding materials. Taken together with the temperature evolution of CBP, this suggests that a real composting process did not occur. Finally, once cows were removed and CBP material was used to build the 2 composting piles, this new process did not lead to any relevant change in CBP materials. In any case, from the agronomic point of view, sawdust and forest biomass present potentially valuable characteristics as regards organic amendment in the soil, thanks to their high organic matter content and low nutrient content.

Key words: compost-bedded pack, dairy cows, forest biomass, organic amendment, sawdust.

4.2. Introduction

Fifty five percent of the world's population lives in urban areas, a proportion that is expected to increase to 68% by 2050 (United Nations, 2018). In this process, the number of both farms and farmers has decreased, thus raising at the same time the urban population (Boogaard et al., 2011). One of the main consequences of this rural flight is an increase in forestland. Human activities have directly caused approximately 60% of the new global tree growth (Xiao-Peng Song et al., 2018). Dry weather and damaged ecosystems with an accumulation of dead biomass due to rural abandonment increase the risk of forest fires. The accumulation of high fuel loads over large areas is the main reason behind the occurrence of large fires (Minnich, 2001; Oliveras et al., 2005). In this context, it is necessary to find new ways to reduce these high fuel loads to prevent large fires.

Compost-bedded pack (CBP) is a loose housing system more and more established on dairy farms, in which cows are maintained for a long time on the compost. Improvements in health, welfare and performance of cows, ease of farm chores and reducing building costs have been described in comparison with other housing systems (Barberg et al., 2007a; Black et al., 2013; Fernández et al., 2020). The composting process allows manure and urine to be stored, as long as the pack is managed adequately, involving twice-daily tilling and periodic bedding addition (Janni et al., 2007; Barberg et al., 2007b; Black et al., 2013). Tilling incorporates manure and air into the pack, thus promoting aerobic microbiological activity, heating the pack and drying the lying surface for cattle to lie on (Shane et al., 2010). Bedding addition increases the water-holding capacity of the pack to control CBP moisture. Compost-bedded pack barns in dairy cow farming mainly use sawdust (S) as bedding material, but forest biomass (FB) could be an alternative material. When both materials were tested as CBP, some important microbial species affecting cow health were better controlled using FB (Llonch et al., 2020).

Once the CBP material is moved away from the barn, it is used as organic fertilizer in the farm. The lack of information about the agronomic value of this fertilizer source led us to study: a) the agronomic characteristics of the CBP obtained either with FB or S, after maintaining cows for three months on the pack, and b) the agronomic characteristics of both composted materials after conducting an additional composting process of three months once the cows were moved away.

4.3. Materials and Methods

Two different composting processes were established. The first involved maintaining cows on CBP, either FB or S, for 11 weeks. Once this one was completed, a second process began, based on composting piles from the two CBPs for 3 months.

4.3.1. Bedding and compost management

Eight dry no pregnant Holstein cows were individually allocated in roofed concrete floor pens, 5 m long and 2.5 m wide. Each pen was divided in 2 areas: a feeding area equipped with a feed bunk and a water trough, and a resting area. Pens were separated by a metal fence that allowed contact between animals. Cows were randomly assigned to 1 of 2 treatments in a cross-over design with 4 cows per treatment. The study was performed in 2 11-week periods with a 4-week washout period between them. Treatments were: 1) CBP with bedding material of S (CBP-S) and 2) CBP with bedding material of FB (CBP-FB). The bulk density for these bedding materials was 182 ± 6.3 g/L and 240 ± 16.2 g/L for S and FB, respectively. During the washout period, cows lay on traditional wood shavings bedding. At the beginning of each period, pens were filled with 30 cm of the new bedding material. All CBP were tilled twice daily (10 am and 5 pm) at 30 cm depth with a rototiller, and an average of 0.8 kg/m^2/d of new bedding material was added on each pen CBP surface before tilling, when pen CBP moisture was greater than 60%. Compost-bedded pack moisture was measured weekly in both areas (feeding and resting) of each pen. An average of 7.8 kg/pen/d in CBP-S and 7.9 kg/pen/d in CBP-FB of new bedding materials were added. Both CBP were removed at the end of week 11 of each period. Daily ambient temperature and environmental humidity were obtained from 2 data loggers (UX100-003, Hobo, Algete-Madrid, Spain) located in the barn. At the end of the second experimental period, the composted bedding material of each pen was composited and used to prepare 2 composting piles, one of each bedding material, to obtain a composted material after 3 months of the composting process. Each pile, in the shape of a truncated pyramid and located in a roofed barn, was 4.8 m long, 2.5 m wide and 1.15 m high, with an approximate volume of 7.5 m^3. The piles were turned and rebuilt weekly to facilitate the aerobic process. It was necessary to water the piles on five occasions to maintain pile moisture between 45 and 65%. Daily ambient temperature and environmental humidity were obtained as described before.

4.3.2. Sampling

Samples were taken from the raw bedding materials, the CBP and the composted piles. Raw bedding material was sampled throughout the experiment. Thus in total, 4 samples of each one were collected and then stored at 4°C until analysis. Particle size of the raw bedding materials was measured using an electromagnetic sieve shaker (RP

200N, CISA Cedaceria Industrial S. L., Barcelona, Spain) to obtain their physical characterization. With regard to the CBP material, temperature was recorded daily and moisture weekly in the feeding and resting areas during each experimental period. Temperature was measured at 15 cm depth with a thermometer and a 15-cm sounding line (K/JR-200+800°C, Ventix, Sant Adrià de Besós, Spain). Sampling for chemical analysis was performed at week 11. Samples were collected at 15 cm depth, from the middle of the feeding and resting area of each pen, and later composited by pen. The amount of sample collected by pen was 5 kg. Samples were stored at -18°C until analysis. Thus, we collected 4 samples per each material and period. Finally, the sampling of the piles was carried out every 10 days, collecting in total 9 samples of 6 kg each one, and then stored at -18°C until analysis. Temperature was measured at 40 cm and 1 m depth with a thermometer and a 1-m sounding line (K/JR-200+800°C, Ventix, Sant Adrià de Besós, Spain). Three samples were taken from each pile in the middle of 3 of the 4 sides of the truncated pyramid, at different depths to obtain a representative sample from each side, and were then composited by pile. The fourth side was next to a wall to facilitate the containment and prevention of landslide, making it inaccessible. In addition, during the composting process, the evolution of wet bulk density, was monitored by weighing material of a known volume, according to Huerta-Pujol et al. (2010). Wet bulk density values were transformed to dry bulk density values by multiplying them by the corresponding dry matter (DM) content of samples.

4.3.3. Chemical analysis

At the moment of analysis, samples were defrosted at room temperature. A watery extract was obtained from each fresh sample using 40 g and 200 mL of distilled water. After 30 min of stirring, the extract was centrifuged at 3600 rpm for 15 min. In the supernatant, the following determinations were carried out: pH and electric conductivity (EC) using a pH meter (Crison GLP 21; Hach Lange Spain, S.L.U., L'Hospitalet de Llobregat, Spain) and a conductivity meter (Crison GLP 31; Hach Lange Spain, S.L.U., L'Hospitalet de Llobregat, Spain), and ammonia nitrogen by means of an ammonium selective electrode (Orion 9512, Termo Fisher Scientific, Barcelona, Spain). Moisture content was determined by drying samples for 24 h at 103ºC. Ash content was determined in the dry and ground (1-mm screen) sample by loss on ignition at 600ºC for 2 h according to AOAC (1990; ID 942.05) to ascertain the organic matter (OM) content of samples. Chemical stability degree (SD) was determined in accordance with López et al. (2010). In dry and ground samples (1-mm), nitrogen content was determined by the Kjeldahl procedure (AOAC, 1990; ID 976.05). The C:N ratio was estimated from the OM and nitrogen content in accordance with Zucconi and de Bertoldi (1987). Mineral nutrient content was determined by flame photometry in the case of K (Model 410, Corning, Halstead, UK) and by spectrophotometry in the case of P (Model Cary 60, Agilent Technologies, Singapore, Malaysia) after dissolution of ash obtained from the ignition of samples at 470°C in 3 N HNO_3.

4.3.4. Statistical analysis

Chemical composition of raw bedding materials was compared using the GLM procedure of SAS (v. 9.3; SAS Institute Inc., Cary, NC, 2011). Data related with the CBP material were analyzed by using the MIXED procedure of SAS (v. 9.3; SAS Institute Inc., Cary, NC, 2011). The model contained the fixed effects of treatment, period and treatment × period interaction, and the random effect of pen nested within sequence, where sequence is the order in which treatment is applied to the experimental unit. The Tukey multiple comparison test was applied to conduct mean separation across treatments and periods when the treatment × period interaction was significant. Regression analyses were performed to obtain the equations and the coefficients of determination between variables studied in the composted piles and the sampling days using the REG procedure of SAS (v. 9.3; SAS Institute Inc., Cary, NC, 2011). The scopes of linear regression, obtained for each composted pile, were compared by means of a *t*-test after checking the homogeneity of variances.

4.4. Results and Discussion

4.4.1. Comparison between bedding materials

Sawdust obtained from sawmills is a bedding material commonly used in CBP barns for dairy cows (Leso et al., 2020; Ferraz et al., 2020), while FB is an alternative material that, in the present study, was composed of tree bark and vegetal fibers from a Mediterranean forest. Both materials showed an acidic pH, a low EC value, high OM content, and low nutrient content, resulting in a high C:N ratio (Table 4.1). These characteristics are common of bedding materials used in CBP, together with a low humidity to assure the absorption capacity of animal urine and a particle size < 25 mm to promote the microbial activity due to the increased growing surface (Shane et al., 2010; Leso et al., 2020). However, certain differences between both materials can be highlighted regarding their physical and chemical properties. Sawdust was slightly acidic with a higher EC value and OM content and lower in humidity and nutrients, resulting in a higher C:N ratio in S than in FB (Table 4.1; $P < 0.001$). In addition, S showed a lower humidity than FB and a finer granulometry, because the proportion of particles < 2 mm was 49.2% and 22.0% for S and FB, respectively.

Table 4.1 Chemical composition and granulometry of raw bedding materials.

Item	Material		SEM	*P*-value
	S[1]	FB[2]		
pH	5.01	6.01	0.112	0.001
EC, mS/cm	0.43	0.23	0.011	0.001
Moisture, %	10.2	32.1	1.97	0.001
OM, % DM	99.1	88.6	0.43	0.001
Stability, %	28.6	52.2	0.22	0.001
Organic N, % DM	0.20	0.35	0.026	0.001
C:N ratio	286	131	20.0	0.001
P, % DM	0.006	0.024	0.0020	0.001
K, % DM	0.054	0.131	0.0088	0.001
Particle size, %				
> 25 mm	0.0	0.0	0.02	0.434
25–12.5 mm	0.6	8.6	0.76	0.001
12.5–10 mm	0.7	6.2	0.50	0.001
10–6.3 mm	8.3	13.2	0.74	0.001
6.3–5 mm	6.8	10.3	1.26	0.077
5–2 mm	34.3	39.7	2.99	0.232
< 2 mm	49.2	22.0	4.62	0.002

[1] S = sawdust.

[2] FB = forest biomass.

To ascertain the chemical SD of the bedding materials, as a way to predict their composting ability, the Klason lignin determination was carried out using the modified method proposed by López et al. (2010). Stability was greater in FB than in S (Table 4.1; $P < 0.001$). Rowell et al. (2012) pointed out that the lignin content of hardwoods (angiosperms) is usually in the range of 18-25%, whereas this content ranges between 25 and 35%, in the case of softwoods (gymnosperms). These higher values can be attributed to the great lignin content of bark pine, between 38 and 58%, an ingredient visually present in FB.

The content of C, N and C:N ratio of the sawdust used in the present experiment was similar to that reported by Shane et al. (2010), but with a P and K content ten times lower. In the case of FB, there is a lack of information with regard to this material when used in CBP barns. Ferraz et al. (2020) described a similar bedding material obtained from the forest (conifer forest litter) to be used in CBP, with similar C and macronutrient contents, and with a C:N ratio more suitable than sawdust for a composting process and greater than 50 to minimize ammonia losses (Kirchmann, 1985). However, the material described by Ferraz et al. (2020) and the FB used in the present experiment differ in their granulometry. In our case, 61.7% of the material was lesser than 5 mm, while this percentage was lower for the conifer forest litter. This, together with the remaining particle fractions, suggests that the forest material described by Ferraz et al. (2020) was more heterogenous and course than FB, probably due to the grinding machinery used. Differences in granulometry explain the differences in bulk density, which were 123 and 240 kg/m^3 for conifer forest litter and FB, respectively.

4.4.2. Comparison between compost-bedded pack (CBP)

4.4.2.1. Temperature and moisture

Temperature and moisture affect the biological transformation of organic materials, particularly in aerobic conditions. Moisture and oxygen availability allow the microbial activity which lead a temperature increase due to exogenous chemical reactions of molecule degradation. In a process such as composting, this temperature increase is observed as an indicator of an adequate evolution. However, in the case of CBP, the objective was not the biological transformation of the material as in the composting but the availability of safe housing for animal breeding. The presence of a C-rich material and the availability of a N source from feces and urine, moisture and oxygen, provide the conditions for microbial growth and, consequently, a temperature increase.

Climatic conditions during the CBP process are shown in Table 4.2. Average ambient temperature was 14.7 ± 3.63°C in Period 1 and decreased from 19.5 to 9.1°C.

Table 4.2 Climatic conditions, temperature and moisture content of compost-bedded pack (CBP) made with sawdust (CBP-S) and forest biomass (CBP-FB) during the time periods studied.

	Period 1	**Period 2**
Climatic conditions		
Temperature, ºC	14.7 ± 3.63	11.2 ± 2.49
Humidity, %	81.5 ± 4.21	74.5 ± 7.18
CBP temperature, ºC		
CBP-S	34.7 ± 5.64	31.2 ± 5.88
CBP-FB	28.3 ± 6.87	25.9 ± 3.08
CBP moisture, %		
CBP-S	54.1 ± 16.97	55.0 ± 16.43
CBP-FB	62.0 ± 14.00	59.4 ± 7.21

In Period 2, average ambient temperature was 11.2 ± 2.49°C and increased from 5.9 to 14.1°C Thus, Period 2 was colder than Period 1, as reflected in the lower temperatures recorded in CBP in Period 2. In Period 1, mean CBP temperature was 34.7°C ± 5.64 in CBP-S and 28.3°C ± 6.87 in CBP-FB, and in Period 2, 31.2°C ± 5.88 and 25.9°C ± 3.08 in CBP-S and CBP-FB, respectively. The lower CBP temperatures recorded in the CBP-FB would indicate a lower microbial activity due to the greater OM stability of this material and bigger particle size. However, in both cases these temperatures were below the recommended range values for an effective composting (NRAES-54, 1992; Janni et al., 2007; Bewley et al., 2013), although this frequently occurs in the context of CBP management (Shane et al., 2010; Leso et al., 2013; Black et al., 2013). Average environmental humidity was 81.5 ± 4.21 in Period 1 and ranged between 74.1 and 90.4%. In Period 2, average environmental humidity was 74.5 ± 7.18% and ranged between 61.6 and 87.3%. In CBP-S the initial moisture was 10% on average and the mean value was 54.1 ± 16.97% in Period 1, and 55.0 ± 16.43% in Period 2. In CBP-FB, the initial moisture was 32% on average and the mean moisture was 62.0 ± 14.00% in Period 1, and 59.4% ± 7.21 in Period 2. A similar moisture content was expected in both CBP, because we decided to add new bedding material when the moisture was greater than 60% applying an average amount of 7.8 kg/d and 7.9 kg/d in CBP-S and CBP-FB, respectively. However, the moisture was slightly higher in CBP-FB than in CBP-S. The humidity and granulometry of FB could also have contributed to this final result.

4.4.2.2. Chemical characteristics

Chemical composition of CBP samples at week 11 is shown in Table 4.3. The pH values recorded were basic and there were increases in the pH and EC values from the raw bedding materials to CBP samples. This increase can be attributed to feces and urine provided by the cows. The pH and EC values were affected by the treatment × period interaction (*P* = 0.001, and *P* = 0.003, respectively). In period 1, CBP-FB pH was higher than CBP-S pH, whereas CBP-FB EC was lower than CBP-S EC. In contrast, CBP-FB pH was lower than CBP-S pH in Period 2, whereas CBP-FB EC was not different from CBP-S EC. The low EC value recorded in CBP-FB in Period 1 could be due to its higher moisture resulting in a higher dilution rate because this determination was made with a wet sample.

Table 4.3 Chemical composition of compost-bedded packs (CBP) after 11 weeks of use.

Item	**Period 1**		**Period 2**		**SEM**	***P*-value**		
	CBP-S[1]	**CBP-FB**[2]	**CBP-S**	**CBP-FB**		**T**	**P**	**T × P**
pH	8.00^{c}	8.43^{b}	8.96^{a}	7.81^{c}	0.078	0.001	0.021	0.001
EC, mS/cm	3.78^{a}	2.39^{b}	3.84^{a}	3.79^{a}	0.163	0.001	0.001	0.003
Moisture, %	61.3^{b}	69.7^{a}	63.7^{b}	63.2^{b}	1.09	0.002	0.037	0.001
OM, % DM	91.1^{a}	83.4^{c}	89.9^{a}	85.7^{b}	0.54	0.001	0.115	0.008
Stability, %	ND	ND	31.7	49.2	1.25	0.001	ND	ND
N, % DM								
Organic	0.89	1.12	1.07	1.21	0.036	0.001	0.002	0.101
Ammonia	0.07	0.08	0.05	0.04	0.014	0.999	0.034	0.444
Total	0.96	1.19	1.12	1.24	0.039	0.001	0.005	0.109
C:N ratio	$48:1^{a}$	$35:1^{c}$	$40:1^{b}$	$35:1^{c}$	1.1	0.001	0.002	0.005
P, % DM	0.24^{b}	0.28^{a}	0.28^{a}	0.25^{ab}	0.009	0.879	0.458	0.003
K, % DM	1.22^{b}	1.64^{a}	1.78^{a}	1.62^{a}	0.098	0.038	0.001	0.015

[1] CBP-S = compost-bedded pack made with sawdust.

[2] CBP-FB = compost-bedded pack made with forest biomass.

The treatment × period interaction also affected OM content (*P* = 0.008). The OM content was higher in CBP-S than in CBP-FB in both periods, but this difference was greater in Period 1 than in Period 2. The content of organic N was affected by treatment (*P* = 0.001), being higher in CBP-FB than in CBP-S. These results could be due to the differences detected in the raw bedding materials where OM was higher in S than in FB, and organic N was higher in FB than in S (Table 4.1). The period also affected organic N (*P* = 0.002), being higher in Period 2 than in Period 1. With regard to ammonia N, this content was affected by period (*P* = 0.034), being greater in Period 1 than in Period 2. However, these differences are not relevant from the agronomic point of view. In addition, there was a treatment × period interaction in the C:N ratio (*P* = 0.005). Although this ratio was greater in CBP-S than in CBP-FB in both periods (*P* = 0.001), in agreement with the differences detected in the raw bedding materials, and it was greater in Period 1 than in Period 2 (*P* = 0.002), the difference between treatments was greater in Period 1 than in Period 2.

The contents of the other macronutrients (P, K) were affected by the treatment × period interaction (*P* = 0.003 and *P* = 0.015, respectively). For the P and K, the content of CBP-FB was greater than in CBP-S in Period 1, but the content between treatments was not different in Period 2. However, considering the higher differences found between raw bedding materials, where the P and K content was higher in FB than in S, the differences between CBPs were lower and in accordance with the raw materials in Period 1, or were not observed in Period 2. In any case, these differences are not relevant from the agronomic point of view.

The pH value, EC, moisture, and the contents of N, P and K increased in CBP samples with regard to the raw bedding materials with high OM content, due to the high macronutrient content supplied by feces and urine. This would explain the C:N ratio decrease in CBP. Thus, final characteristics of CBP will be linked with the bedding material added throughout the process. Taking into account the amount added (7.8 kg/d and 7.9 kg/d in CBP-S and CBP-FB, respectively) and the bulk density of the bedding material, the amounts added were 15.6 m^3/cow/year and 12.0 m^3/cow/year, for CBP-S and CBP-FB, respectively. These values are inside the range reviewed by Leso et al. (2020). In addition, the chemical characteristics found in the present experiment for CBP-S are in the range found by Shane et al. (2010) and Eckelkamp et al. (2016) for wood shavings and wood chips.

The SD of the OM in CBP, measured in Period 2, was greater in CBP-FB than in CBP-S, in agreement with the differences recorded in the raw bedding materials. The SD measured in CBP-S remained far away from 50, the threshold used to consider a compost mature. Taken together with the temperature evolution of CBP, this suggests that there was not a real composting process.

4.4.3. Comparison between the composted piles

4.4.3.1. Temperature, moisture and bulk density

From the beginning to the end of the composting period, ambient temperature increased from 22 to 26°C and environmental humidity from 47 to 72%. The average initial temperatures of the composting piles were 35.0°C and 30.4°C for S and FB, respectively, the average final temperatures being 46.0°C and 44.5°C (Figure 4.1a). In both cases, a temperature increase was observed during the composting process achieving the thermophile phase (> 40°C), and reaching hygienization temperature for S (> 55°C more than 15 d) while FB remained below this limit. Temperature achieved at d 90 in both piles would indicate that the composting process had not ended, probably due to the low C:N ratio and the lignocellulosic content of both materials, in particular in FB. The initial moisture of the S pile and the FB pile was 61.5% and 62.2%, respectively, whereas the final moisture was 47.9% and 55.4% (Figure 4.1b). The values over the period were adequate for a composting process, being over 50% in order to maintain microbial activity (Haug, 1993). The piles were watered periodically, which helped to conserve the moisture. The bulk density evolution is shown in Figure 4.2. Dry bulk density increased slightly, and changes were due to mineralization and particle size reduction. Bulk density was higher in the FB pile than in S pile.

Figure 4.1 Effect of treatment (sawdust = S and, forest biomass = FB) on temperature (a) and moisture (b) of the composted piles.

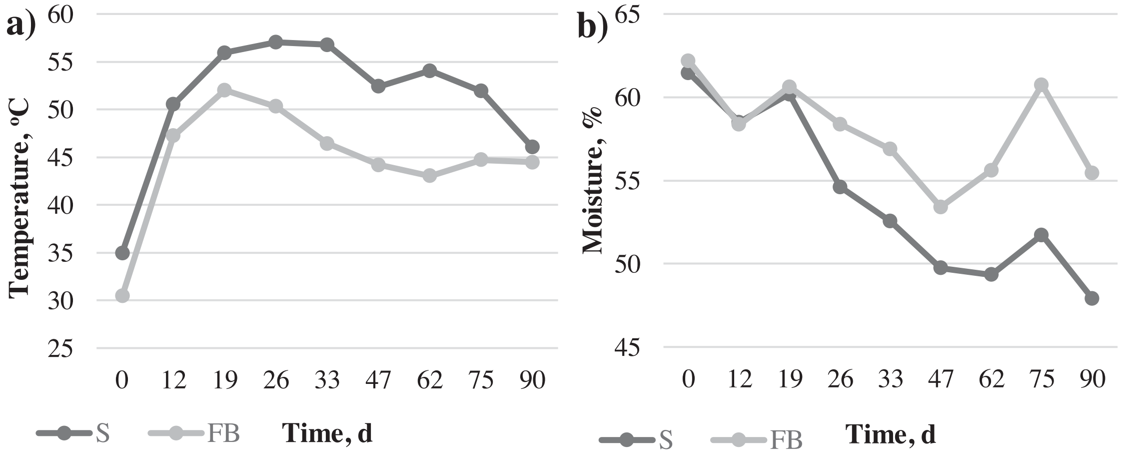
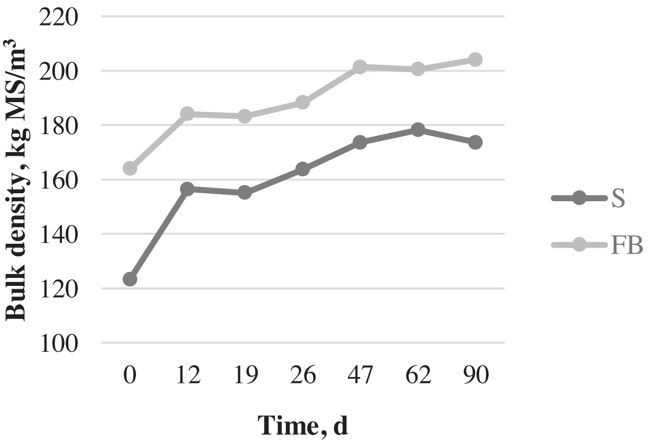


Figure 4.2 Bulk density evolution of samples taken from the composted piles (sawdust = S and forest biomass = FB).



4.4.3.2. Chemical characteristics

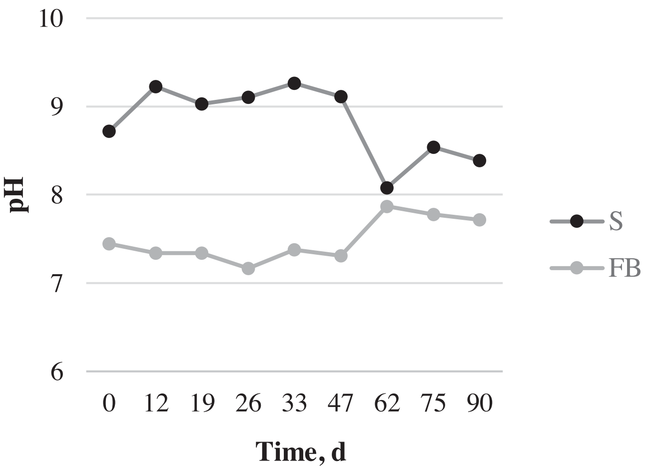
Initial and final values of the variables measured in the piles are shown in Table 4.4. In a composting process, an initial acidification followed by a gradual alkalinization is a common evolution of the pH medium. However, in the present experiment, the changes in the pH values of the piles were small and did not respond to this pattern (Figure 4.3). The pH values were more basic in S than in FB with the corresponding greater risk of N losses in S. With regard to EC, an increase in its value was expected during the composting process due to OM mineralization. This happened in S but not in FB, proving that composting was less strong in the FB pile.

Although a small decrease in the OM in both piles was observed, the content was high in both cases at d 90, which would indicate only a partial transformation of this OM during composting. This could be explained by the high C:N ratio recorded at d 0, which reflected a N lack, and by the high presence of lignin in wood waste, which would explain the difficulty in the OM degradation. Thus, at the end of the process, a large quantity of OM remained non degraded. Nevertheless, a slight increase in stability

Table 4.4 Initial and final values (Mean ± sd) of the variables measured in the composted piles made with sawdust (S) and forest biomass (FB).

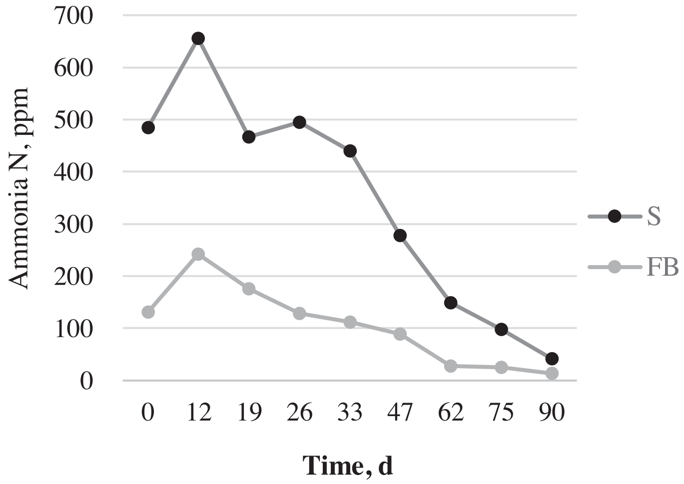
Item	S		FB	
	Day 0	**Day 90**	**Day 0**	**Day 90**
pH	8.72 ± 0.017	8.39 ± 0.035	7.45 ± 0.015	7.72 ± 0.100
EC, mS/cm	3.59 ± 0.145	5.58 ± 0.106	3.39 ± 0.025	3.10 ± 0.061
Moisture, %	61.5 ± 0.24	47.9 ± 0.15	62.2 ± 0.29	55.4 ± 0.18
OM, % DM	91.7 ± 0.18	87.4 ± 0.13	86.3 ± 0.30	82.9 ± 0.03
Stability, %	31.7	34.9	49.2	53.9
N, % DM				
Organic	0.88 ± 0.039	1.05 ± 0.058	1.16 ± 0.013	1.27 ± 0.054
Ammonia	0.049 ± 0.0004	0.004 ± 0.0002	0.013 ± 0.0006	0.001 ± 0.0005
C:N ratio	52.0	41.8	37.2	32.6
P, % DM	0.24 ± 0.017	0.31 ± 0.040	0.27 ± 0.021	0.24 ± 0.011
K, % DM	1.74 ± 0.071	2.02 ± 0.134	1.94 ± 0.269	1.87 ± 0.066

Figure 4.3 Evolution of pH of samples taken from the composted piles (sawdust = S and forest biomass = FB).



was observed, from 31.7 to 34.9% and 49.2 to 53.9%, respectively, for S and FB. According to López et al. (2010), the value over 50% recorded in the FB pile at d 90, would indicate that the material was chemically stable, and would produce a progressive degradation of OM in an eventual soil application and a consequent gradual release of nutrients. There was a slight increase in the content of N, P and K from d 0 to d 90 in the S pile, which can be explained by a concentration process due to the OM decrease. In the FB pile, this slight increase was only observed in N content. The initial ammonia N content was low in both piles, in agreement with their low total N content. Although it was lower in FB than in S, its evolution over time was similar to that normally observed in a composting process because after a slight increase during the first days, it then decreased till d 90 (Figure 4.4). This decrease could be explained by utilization by microorganisms, the nitrification process or the loss caused by turning (DeLaune et al., 2004). All these values were close to or below the upper limit proposed by Zucconi and de Bertoli (1987) to consider compost being mature.

Figure 4. 4 Evolution of ammonia N content of samples taken from the composted piles (sawdust = S and forest biomass = FB).



In order to accelerate the composting process, it would be necessary to increase the N content in the original material by adding a N source to reduce its initial C:N ratio. In this sense, the practice used by some farmers of drying the material resulting in the CBP and reusing it in the barn could help achieve this objective. However, effort should be made to achieve the appropriate conditions to assure the hygenization of material and avoid animal health problems. Another alternative to achieve this hygenization, when lignocellulosic materials are used, could be to lengthen the process (> 6 months).

The progression of each chemical compound of these piles over time, expressed with the corresponding regression equation, is shown in Table 4.5. The relationship was statistically significant in both piles for OM, organic N, ammonia N and C:N ratio, the slope being negative for OM, ammonia N and C:N ratio, and positive for organic N. In those variables, the slope comparison showed that there were no statistical differences between treatments (data not shown). Moreover, in the sawdust pile the relationship exists also for EC, moisture, and total N, and in forest biomass pile, for pH.

Table 4.5 Regression equations in each composting pile (sawdust = S; forest biomass = FB) between chemical variables and time (d) of composting period.

Item	S				FB			
	Equation	R^2	*P*-value	RMSE	Equation	R^2	*P*-value	RMSE
pH	9.19-0.009d	0.41	0.064	0.343	7.25+0.006d	0.53	0.026	0.178
EC, mS/cm	3.66+0.023d	0.95	0.001	0.176	2.03+0.001d	0.02	0.745	0.252
Moisture,%	59.9-0.146d	0.79	0.002	2.468	59.9-0.047d	0.25	0.174	2.681
OM, % DM	91.0-0.041d	0.90	0.001	0.499	85.3-0.027d	0.65	0.028	0.688
N, % DM								
Organic	0.88+0.003d	0.68	0.006	0.061	1.14+0.001d	0.48	0.039	0.037
Ammonia	0.06-0.001d	0.89	0.001	0.008	0.02-0.001d	0.75	0.002	0.004
C:N	52.0-0.145d	0.84	0.004	2.286	37.5-0.050d	0.73	0.015	1.073
P, % DM	0.26+0.001d	0.63	0.107	0.019	0.28-0.001d	0.27	0.292	0.028
K, % DM	1.83+0.004d	0.71	0.073	0.107	1.76+0.002d	0.29	0.270	0.127

4.4.4. Agronomic suitability of the materials

Both CBP materials contain high amounts of OM and a low nutrient content in comparison with cattle manure. Thus, they must be considered organic fertilizers to provide OM to the soil. Leso et al. (2020), in their CBP review, regard this material as a green waste compost, and stated that in the long term the use of CBP as manure can result in considerably higher amounts of OM and a larger accumulation of N than cattle manure. The C:N ratio of both CBP was high but within the wide range, from 10.5 to 49.3, reviewed by Leso et al. (2020). In addition, high ratios are expected when wood-derived materials are considered (Shane et al., 2010). The incorporation in the soil of materials with a high C:N ratio, can initially lead to a N inorganic immobilization, because it is used by microbes, making it temporarily inaccessible to crops. This negative effect could happen if the material of the S pile was used due to its less stable OM, but not in the case of FB pile material with a more stable OM. However, in accordance the Spanish regulation RD506/2013 on fertilizing, both materials would be unmarketable because of a C:N ratio greater than 20. Thus, this final composting process did not bring about any meaningful change to CBP materials. Only if hygienization was achieved could this additional work be justified to add safety in cattle farms.

4.5. Conclusions

From the agronomic point of view, both materials present potentially valuable characteristics when it comes to organically amending the soil, as they are high in organic matter content and low in nutrients. Also, the organic matter of forest biomass pile was well stabilized (more than 50%). Nevertheless, some improvement in the composting management can still be made to achieve effective composting, assuring an adequate C:N ratio and the hygienization of this material in the case of its reutilization as compost-bedded pack.

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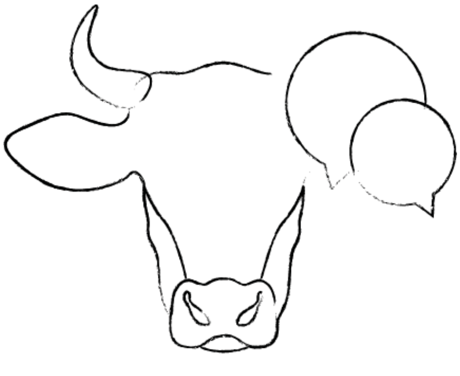

CHAPTER 5.

General Discussion

The issues discussed in the present doctoral thesis can be classified according to the conceptualization of Fraser et al. (1997). In the first study, the lack of an optimal peNDF supply in beef cattle involving a potential type 3 problem, SARA. Biological functioning of the rumen would be affected by the feeding system, and animals would sort the feed to reduce the discomfort caused by this disease. In the second study, the lack of information on forest biomass as bedding material in CBP involving potential type 3 problems, such as lameness or mastitis. Biological functioning of legs and udder would be affected by the housing system, and animals would modify their behavior to reduce the discomfort caused by these injuries.

Regarding these issues, we studied management strategies that modified the housing and feeding system with the aim of making these environments more suitable for animal needs and to avoid or correct type 3 problems. The success of these strategies was based on the ability and efforts of the animal to cope with the modified environment. These strategies could be considered indirect indicators of cattle welfare because they provide information about how risky the environment is for the animal. In the first study, the feed composition was the indirect indicator of the feeding system, and the peNDF content of the diet was the management-based measure which regards procedures used to protect animals from diseases. In the second study, the resting area was the indirect indicator of the housing system, and the bedding material was the resource-based measure which regards the environment in which the animals are kept. As indirect indicators need animal-based measures to be validated and established, the assessment of these strategies used a set of direct indicators (behavior, health and physiology) linked to animal-based measures. In the second study, indirect indicators linked to resource-based measures were also used. The assessment goal was the provision of welfare assurance in the research context.

The establishment of these management strategies as indirect indicators must follow a validation protocol, but this study could be considered a first approach in the search for results of these strategies. Besides the individual assessment of the animal-based, resource-based and management-based measures made in both papers, a more complex study of these measures may help to clarify the results of these strategies. The aim of this chapter was to reanalyze some of the data obtained in both experiments and to discuss them again from other perspective. In addition, because some additional data were collected in the first experiment, this information was added and discussed.

5.1. Feeding System in Beef Cattle

5.1.1. Correlation coefficients and regression analysis of variables linked to physically effective fiber (peNDF)

Pearson correlation coefficients among variables linked to peNDF were obtained using the CORR procedure of SAS (Table 5.1). Coefficients were declared significant at $P < 0.05$ and trends were discussed at $P < 0.10$. Data used in each variable were the mean week per period and animal, resulting in 32 observations per variable. Variables chosen were those measured directly or with least possible transformation.

Dietary peNDF correlated positively with intake of peNDF ($r = 0.96$, $P < 0.01$) and time spent ruminating ($r = 0.65$, $P < 0.01$). Intake of peNDF correlated positively with ruminating ($r = 0.60$, $P < 0.01$). These positive correlations were consistent with the linear increase in intake of peNDF and ruminating with increasing dietary peNDF

Table 5.1 Pearson correlation coefficients[1] among variables linked to peNDF in beef heifers fed a high-concentrate diet.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. Body weight, kg																				
2. Dietary peNDF[2], % DM	-0.27																			
Intake																				
3. DM, kg/d	0.80	-0.40																		
4. NDF, kg DM/d	0.56	-0.34	0.81																	
5. Physically effective NDF, % DMI[3]	-0.32	0.96	-0.36	-0.29																
6. Water consumption, L/d	0.87	-0.27	0.80	0.62	-0.29															
7. Extent of sorting of particles >4mm, %	-0.05	-0.63	0.29	0.32	-0.39	0.01														
8. Meal length, min/meal	-0.09	-0.19	0.09	-0.01	-0.13	-0.06	0.28													
9. Meal size, g/meal	0.21	-0.06	0.02	0.00	-0.07	0.02	0.02	-0.30												
10. Time spent ruminating, min/d	-0.05	0.65	-0.16	-0.29	0.60	-0.16	-0.55	0.11	0.03											
Rumen pH																				
11. Mean	0.15	0.03	0.13	0.06	0.00	-0.03	-0.09	0.35	-0.23	0.22										
12. Min	0.21	-0.07	0.31	0.14	-0.02	0.05	0.18	0.52	-0.17	0.18	0.84									
13. Max	-0.11	0.07	-0.25	-0.17	-0.01	-0.16	-0.25	0.14	-0.14	0.07	0.72	0.33								
Duration, h/d																				
14. pH < 5.8	-0.26	-0.13	-0.27	-0.13	-0.10	-0.05	0.17	-0.26	0.11	-0.28	-0.72	-0.70	-0.25							
15. pH < 5.7	-0.25	-0.13	-0.26	-0.14	-0.18	-0.08	0.20	-0.21	0.18	-0.22	-0.66	-0.63	-0.21	0.98						
16. pH < 5.6	-0.25	-0.13	-0.27	-0.17	-0.09	-0.11	0.19	-0.15	0.20	-0.15	-0.61	-0.56	-0.18	0.94	0.98					
17. pH < 5.5	-0.29	-0.16	-0.29	-0.20	-0.12	-0.17	0.20	-0.06	0.21	-0.07	-0.56	-0.49	-0.20	0.84	0.91	0.96				
AUC[4], pH×h/d																				
18. pH < 5.8	-0.23	-0.04	-0.23	-0.17	-0.05	-0.04	0.01	-0.34	0.04	-0.25	-0.86	-0.81	-0.52	0.86	0.81	0.75	0.69			
19. pH < 5.7	-0.23	-0.05	-0.24	-0.19	-0.02	-0.05	0.02	-0.28	0.09	-0.17	-0.82	-0.76	-0.49	0.87	0.85	0.82	0.79	0.97		
20. pH < 5.6	-0.25	-0.02	-0.26	-0.23	-0.04	-0.08	-0.02	-0.22	0.08	-0.07	-0.77	-0.70	-0.48	0.83	0.83	0.83	0.83	0.93	0.98	
21. pH < 5.5	-0.33	-0.06	-0.27	-0.26	-0.02	-0.19	0.03	-0.08	0.08	0.01	-0.69	-0.57	-0.48	0.71	0.73	0.75	0.82	0.84	0.91	0.95

[1] Correlation coefficients were significant at $P < 0.01$ (> 0.48 or < -0.48), $P < 0.05$ (> 0.34 or < -0.34), and $P < 0.10$ (> 0.29 or < -0.29).

[2] Physically effective neutral detergent fiber proportion in the diets (DM basis).

[3] Intake of peNDF (kg DM/d) divided by intake of DM (kg/d).

levels in the present study (Chapter 3.1), suggesting that increasing dietary peNDF stimulated intake of peNDF, and consequently, rumination activity. The lack of correlation among variables like dietary peNDF, intake of peNDF and time spent ruminating, and variables related to ruminal pH like mean, minimum, maximum rumen pH and area under the curve (AUC) of critical rumen pH thresholds, corresponds with the lack of linear effect observed in these variables as dietary peNDF increased (Chapter 3.1). However, the linear effect was detected in the duration under critical rumen pH thresholds. In this case, the lack of correlation could be explained by the variability in ruminal pH among animals, even when they were fed the same diet (Bevans et al., 2005). Regarding the literature, the lack of correlations between dietary peNDF and ruminal pH variables in the present study was in agreement with Beauchemin and Yang (2005) and Yang and Beauchemin (2006), but contrast with the findings of Yang and Beauchemin (2009). However, the correlation between time spent ruminating and dietary peNDF in the present study is consistent with most studies (Beauchemin et al., 2003; Yang and Beauchemin, 2006; Yang and Beauchemin, 2009). This suggested that ruminal pH, unlike rumination, was affected by other factors not explained by peNDF, such as ruminal fermentability of feeds. Overall, the increase in dietary peNDF content stimulated rumination activity, which is a natural behavior in cattle necessary for their welfare and ruminal health.

Dietary peNDF and intake of peNDF correlated negatively with DMI ($r = -0.40$, $P < 0.05$, and $r = -0.36$, $P < 0.05$, respectively) and extent of sorting of particles > 4 mm ($r = -0.63$, $P < 0.01$, and $r = -0.39$, $P < 0.05$, respectively). These negative correlations were consistent with the linear decrease in DMI and extent of sorting of particles > 4 mm with increasing dietary peNDF levels in the present study (Chapter 3.1), suggesting that increasing dietary peNDF reduced intake and led to sorting against particles > 4 mm. Intake of peNDF tended to correlate negatively with BW (r = -0.32, $P < 0.10$), and dietary peNDF tended to correlate negatively with intake of NDF (r = -0.34, $P < 0.10$). These negative coefficients reflect negative consequences for cattle production, because they reduce animal performance, and for cattle ruminal health, because they promote sorting against particles which stimulate rumination activity. Thus, the increase in dietary peNDF content must be at a level which avoids affecting animal production and ruminal health.

With regard to ruminal pH variables, meal length correlated positively with mean pH (r = 0.35, $P < 0.05$) and minimum pH (r = 0.52, $P < 0.01$), and also tended to correlate negatively with AUC of pH < 5.8 (r = -0.34, $P < 0.10$). This suggested that a longer time spent per meal reduced the risk of SARA by increasing mean pH and minimum pH and potentially reducing AUC of pH < 5.8. In the present study, different dietary peNDF levels promoted similar meal lengths (Chapter 3.1), indicating that dietary peNDF would not be a useful tool to modify meal length. As animals were individually allocated, this correlation seemed to be caused by individual variability in feeding behavior. Further studies should examine this individual variability in similar conditions in order to implement strategies to better control meal length.

Increasing dietary peNDF did not affect mean and minimum rumen pH, and AUC of all critical pH thresholds, but linearly decreased the number of hours under these critical thresholds, so these durations were considered the main representative indicator to detect a response to the increased peNDF in the diet (Chapter 3.1). However, when coefficients of correlations among rumen pH variables were obtained, high values were found. Mean pH correlated positively with minimum pH (r = 0.84, $P < 0.01$) and maximum pH (r = 0.72, $P < 0.01$); mean pH and minimum pH correlated negatively with the duration of all critical pH thresholds (r between -0.49 to -0.72, $P < 0.01$); mean pH, minimum pH and maximum pH correlated negatively with AUC of all critical pH thresholds (r between -0.48 to -0.86, $P < 0.01$); duration of all critical pH thresholds and AUC of all critical pH thresholds correlated positively among each other (r between 0.71 to 0.98, $P < 0.01$); and minimum pH tended to correlate positively with maximum pH ($r = 0.33$, $P < 0.10$). In summary, the higher the mean and minimum rumen pH, the lower is the number of hours or the AUC of critical thresholds, as expected.

The data recorded in this experiment (Chapter 3.1) offered us a good opportunity to ascertain whether some variables considered relevant from the animal performance and welfare point of view (peNDF intake, sorting behavior, minimum pH, etc.) could be predicted with independent variables by multiple regression analysis (Table 5.2). Intake of DM, as dependent variable, was assessed using the REG procedure of SAS with dietary peNDF, BW, water consumption and ruminating as independent variables. Intake of peNDF and extent of sorting of particles > 4 mm, and ruminal pH parameters, as dependent variables, were assessed using the REG procedure of SAS with dietary peNDF, BW, DMI, intake of NDF, water consumption, ruminating, meal length and meal size as independent variables. To limit overparameterization of the model, a variance inflation factor (VIF) less than 10 for every continuous independent variable tested was assumed, as suggested by Myers (1990). The multiple regression analyses that were significant ($P < 0.05$) were further tested using forward, backward and stepwise elimination multiple regression. The best-fit equation of multiple regression in each dependent variable was chosen as the one with the highest determination coefficient (R^2), lowest root mean square error (RMSE), lowest conceptual predictive criteria (Cp) statistic, and lowest predicted residual error sum of squares (PRESS)

Table 5.2 Multiple regression[1] of responses of intake and rumen pH variables to different measurable on-farm factors in beef heifers fed high-concentrate diet.

Item (Y)	Factor[2] (X)	Parameter estimates				Model statistics	
		Intercept	SE	Slope	SE	RMSE[3]	R^2
Intake							
DM, kg/d	WC, L/d	2.44	1.07	0.109	0.051	0.62	0.72
	peNDF, % DM			-0.053	0.029		
	BW, kg			0.010	0.005		
Physically effective NDF, % DMI[4]	peNDF, % DM	1.92	1.44	0.806	0.040	0.80	0.94
	DMI, kg/d			0.635	0.228		
	BW, kg			-0.017	0.006		
Extent of sorting of particles > 4 mm, %	DMI, kg/d	119	21.4	5.070	2.027	6.38	0.57
	peNDF, % DM			-1.329	0.399		
	BW, kg			-0.147	0.052		
Rumen pH							
Mean	WC, L/d	5.45	0.62	-0.049	0.018	0.21	0.36
	ML, min/meal			0.021	0.011		
	BW, kg			0.006	0.002		
	MS, g/meal			-0.0004	0.0002		
Minimum	DMI, %DM	2.45	0.79	0.145	0.079	0.25	0.50
	WC, L/d			-0.059	0.023		
	ML, min/meal			0.050	0.014		
	peNDF, % DM			0.015	0.013		
	BW, kg			0.005	0.002		
Duration, h/d							
pH < 5.8	DMI, % DM	8.71	2.98	-0.690	0.458	1.56	0.38
	WC, L/d			0.395	0.146		
	BW, kg			-0.035	0.016		
	R, min/d			-0.006	0.004		
	MS, g/meal			0.002	0.001		
pH < 5.7	WC, L/d	3.49	1.60	0.264	0.079	0.40	0.26
	BW, kg			-0.034	0.008		
	MS, g/meal			0.002	0.001		
pH < 5.6	DMI, % DM	4.54	1.83	-0.427	0.288	0.93	0.33
	WC, L/d			0.203	0.086		
	peNDF, % DM			-0.075	0.047		
	BW, kg			-0.019	0.009		
	MS, g/meal			0.002	0.001		
AUC[5], pH × h/d							
pH < 5.8	WC, L/d	11.5	3.24	0.219	0.097	1.77	0.31
	ML, min/meal			-0.154	0.062		
	BW, kg			-0.028	0.010		

[1] Best-fit equations of multiple regressions (forward or backward elimination) are shown.

[2] peNDF = dietary physically effective NDF; BW = body weight; DMI = dry matter intake; WC = water consumption; R = time spent ruminating; ML = meal length; MS = meal size.

[3] Root mean square error.

[4] Intake of peNDF (kg DM/d) divided by intake of DM (kg/d).

[5] Area under the curve of rumen pH.

statistic. The best-fit equations fell under forward or backward elimination procedure, used depending on each dependent variable. In the forward elimination procedure, significance for entering was set at 0.25, in accordance with Myers (1990), who recommended values between 0.25 and 0.50. In the backward elimination procedure, significance for remaining was set at 0.10, in accordance with Myers (1990), who recommended values between 0.01 and 0.10. As in the correlation assessment, data in each variable were the mean week per period and animal, resulting in 32 observations per variable.

Dependent variables were chosen because of their usefulness to prevent SARA, their correlation with dietary peNDF and their low feasibility to be measured on farm, whereas independent variables were chosen because of their correlation with dietary peNDF and their better feasibility to be measured on farm. Some of these independent variables are not commonly measured on fattening cattle farms, such as time spent ruminating or feeding behavior, but taking into account the smart-farming future, they could be implemented on farms.

Maximum rumen pH, duration of pH < 5.5, and AUC of pH < 5.7, < 5.6 and < 5.5, were non-significant ($P > 0.05$) in the multiple regression analysis (data not shown). This means that these dependent variables cannot be predicted with the chosen independent variables in the present study.

Best-fit equations of minimum rumen pH, duration of pH < 5.8 and < 5.6, and AUC of pH < 5.8 used the forward elimination procedure, and best-fit equations of DMI, intake of peNDF, extent of sorting of particles > 4 mm, mean rumen pH and duration of pH $< 5{,}7$ used the backward elimination procedure.

Intake of DM was predicted with a coefficient of determination of 0.72. It was positively affected by water consumption and BW, and negatively by dietary peNDF, in agreement with previous correlations. Intake of peNDF and extent of sorting of particles > 4 mm were predicted ($R^2 = 0.94$ and $R^2 = 0.57$, respectively), both being positively affected by DMI and negatively by BW, and positively affected by dietary peNDF, in the case of intake of peNDF, and negatively affected by dietary peNDF, in the case of extent of sorting of particles > 4 mm, in agreement with previous correlations. Intake of DM and peNDF were well predicted using independent variables easy to measure on farm (dietary peNDF, body weigth) or variables that rely on technological tools to be measured (water consumption, DMI). Tafaj et al. (2007) predicted the feed intake (kg DM/day or DMI) in lactating dairy cows with a lower coefficient of determination ($R^2 = 0.33$) than in the present study, but only used the dietary factor of NDF to make the prediction. Interestingly, intake of peNDF could be accurately predicted with few and feasible variables such as dietary peNDF, DMI and BW, estimating the effect of sorting behavior to make possible changes in feed formulation or management.

Mean rumen pH was predicted with a coefficient of determination of 0.36, being positively affected by meal length and BW, and negatively by water consumption and meal size. Minimum rumen pH was predicted with a $R^2 = 0.50$, being positively affected by DMI, meal length, dietary peNDF and BW, and negatively by water consumption. Duration of pH < 5.8 was predicted with a coefficient of determination of 0.38, and was positively affected by water consumption and meal size, and negatively by DMI, BW and ruminating. Duration of pH < 5.7 was predicted with a $R^2 = 0.26$, being positively affected by water consumption and meal size, and negatively by BW. Duration of pH < 5.6 was predicted with a $R^2 = 0.33$, and was positively affected by water consumption and meal size, and negatively by DMI, dietary peNDF and BW.

Area under the curve of pH < 5.8 was predicted with a coefficient of 0.31, being positively affected by water consumption, and negatively by meal length and BW. Pearson correlation values previously obtained are in agreement with the independent variables used in these equations. Zebeli et al. (2006) predicted rumen pH of lactating dairy cows with two equations, both with higher coefficient of determinations (R^2 = 0.72 and R^2 = 0.75) than in the present study, using dietary peNDF and rumen fermentation parameters as independent variables. Tafaj et al. (2007) reported a moderate coefficient of determination (R^2 = 0.55) to predict rumen pH by particle size and dietary NDF, variables used to calculate dietary peNDF. González et al. (2012) predicted rumen pH of feedlot cattle with a high coefficient of determination (R^2 = 0.80), using the sodium bicarbonate concentration in the concentrate, DMI, meal size and chewing time, where meal size played an important role. In the present study, measurements like BW, water consumption and DMI played a more important role as independent variables than dietary peNDF or time spent ruminating in most ruminal pH prediction equations, lending feasibility though low accuracy to the prediction. Overall, the best equation to predict rumen disorders was achieved in the case of minimum pH, in which the independent variables chosen by the regression analysis explained 50% of its variation. In further studies, the inclusion of other variables linked to the fermentative characteristics of the diet in the prediction of ruminal pH would increase accuracy.

5.2. Housing System in Dairy Cows

5.2.1. Relationship between compost-bedded pack (CBP) performance and weather conditions

Prediction equations of CBP performance based on weather conditions were statistically obtained by regression, using the REG procedure of SAS (Table 5.3). Equations of CBP temperature were obtained using the pen mean of daily CBP temperature, and the corresponding daily ambient temperature and environmental humidity. Equations of CBP moisture and C:N ratio were obtained using the pen mean of weekly CBP moisture and C:N ratio, and the corresponding weekly mean ambient temperature and environmental humidity. Because environmental humidity did not predict ($P > 0.05$) the variables related with the CBP performance, data are not shown.

Ambient temperature affected CBP performance in the present study, in agreement with Eckelkamp et al. (2014). Thus, the ambient temperature could be a predictor of CBP performance because R^2 showed significant *P*-values. With regard to CBP temperature, ambient temperature in period 1 predicted CBP temperature of both treatments with moderate R^2 values, unlike low R^2 values in period 2. This suggested that CBP temperature was better predicted in period 1, when ambient temperature decreased, than in period 2. Similarly to Black et al. (2013) and Eckelkamp et al. (2014), CBP temperature recorded in the present study increased as ambient temperature increased in both periods. The coefficients of determination were higher in CBP-FB than in CBP-S in both periods, suggesting that ambient temperature explained a greater proportion of CBP temperature variance in CBP-FB than in CBP-S. Regarding CBP moisture, the proportion of variance explained by ambient temperature was higher in period 2 than in period 1, suggesting that CBP moisture was better predicted when

Table 5. 3 Simple regression between the CBP performance dependent variables and the ambient temperature (independent variable).

Item (Y)	Parameter estimates				Model statistics[3]	
	Intercept	SE	Slope	SE	RMSE	R^2
CBP-S[1]						
Period 1						
CBP Temperature	19.3	1.04	1.045	0.069	4.60	0.43
CBP Moisture	83.8	6.33	-1.950	0.429	11.17	0.34
CBP C:N ratio	-222	9.24	28.637	0.725	7.75	0.99
Period 2						
CBP Temperature	25.0	1.66	0.585	0.141	5.95	0.06
CBP Moisture	20.2	4.92	3.252	0.418	8.24	0.60
CBP C:N ratio	398	51.1	-24.151	4.336	46.7	0.80
CBP-FB[2]						
Period 1						
CBP Temperature	8.21	0.91	1.358	0.061	4.03	0.63
CBP Moisture	86.8	6.44	-1.571	0.428	9.72	0.25
CBP C:N ratio	-33.1	32.1	9.430	2.516	26.9	0.64
Period 2						
CBP Temperature	19.3	0.91	0.604	0.078	3.28	0.17
CBP Moisture	44.4	2.49	1.401	0.212	4.17	0.52
CBP C:N ratio	208	19.0	-11.118	1.612	17.3	0.86

[1]CBP-S = Compost-bedded pack of sawdust treatment.
[2]CBP-FB = Compost-bedded pack of forest biomass treatment.
[3]*P*-values for coefficients of determination = $P < 0.01$.

ambient temperature increased. Conversely to Eckelkamp et al. (2014), CBP moisture in the present study increased as ambient temperature increased in period 2 and vice versa in period 1, albeit not consistently. The coefficients of determination were higher in CBP-S than in CBP-FB in both periods, suggesting that ambient temperature provided a better estimation of CBP moisture in CBP-S than in CBP-FB. With regard to CBP C:N ratio, ambient temperature predicted CBP C:N ratio of both treatments in both periods, with high R^2 values. This suggested that CBP C:N ratio was accurately predicted in both periods, regardless of ambient temperature pattern, but CBP C:N ratio increased as ambient temperature increased in period 1 and vice versa in period 2, albeit not consistently. The proportion of variance explained by ambient temperature was higher in CBP-S than in CBP-FB in period 1, but was more similar between treatments in period 2. In summary, the proportion of the variance in CBP performance variables explained by the ambient temperature showed an increasingly more accurate scale, from CBP temperature, with lower coefficients of determination, CBP moisture to CBP C:N ratio, with higher coefficients. However, RMSE values, which reflect the dispersion of predicted values from real values, decreased from CBP C:N ratio, CBP moisture, to CBP temperature. A possible explanation for the results obtained, especially in relation to the discrepancies between periods, could be linked to the relatively short period in the composting process (less than 3 months) and the standard pattern of the pack at the beginning of the CBP system establishment. Overall, CBP temperature with period 1 conditions was accurately predicted with ambient temperature. In addition, although CBP moisture with period 2 conditions was accurately predicted with ambient temperature, and CBP C:N ratio was accurately predicted with ambient temperature, discrepancies between periods suggested that factors other than weather conditions could have affected the relationship between CBP and weather conditions.

5.2.2. Effect of the composting time on compost-bedded pack (CBP) performance

Prediction equations of CBP performance based on pack composting days were statistically obtained by regression, using the REG procedure of SAS (Table 5.4). Equations of CBP temperature were obtained using the pen mean of daily CBP temperature and the corresponding pack composting day. Equations of CBP moisture and CBP C:N ratio were obtained using the pen mean of weekly CBP moisture and CBP C:N ratio, and the corresponding pack composting day.

Table 5.4 Simple regression between the CBP performance dependent variables and the composting day (independent variable).

Item (Y)	**Parameter estimates**				**Model statistics**[3]	
	Intercept	**SE**	**Slope**	**SE**	**RMSE**	**R^2**
CBP-S[1]						
Period 1						
CBP Temperature	42.8	0.50	-0.207	0.011	4.16	0.54
CBP Moisture	36.5	2.71	0.497	0.060	8.37	0.63
CBP C:N ratio	303	11.3	-3.637	0.208	17.3	0.97
Period 2						
CBP Temperature	27.8	0.72	0.096	0.016	5.77	0.11
CBP Moisture	41.2	3.10	0.404	0.069	9.58	0.46
CBP C:N ratio	273	30.6	-3.110	0.565	47.07	0.79
CBP-FB[2]						
Period 1						
CBP Temperature	38.8	0.37	-0.271	0.008	3.01	0.79
CBP Moisture	49.1	2.51	0.371	0.056	7.74	0.53
CBP C:N ratio	146	13.5	-1.343	0.249	20.76	0.78
Period 2						
CBP Temperature	23.2	0.40	0.073	0.009	3.24	0.19
CBP Moisture	53.2	1.48	0.180	0.033	4.57	0.43
CBP C:N ratio	149	12.1	-1.416	0.224	18.64	0.83

[1] CBP-S = Compost-bedded pack of sawdust treatment.

[2] CBP-FB = Compost-bedded pack of forest biomass treatment.

[3] P-values for coefficients of determination = $P < 0.01$.

Regarding CBP performance depending on pack composting days, all *P*-values of regression assessment were significant. As for CBP temperature, pack composting days in period 1 predicted CBP-S temperature with lower R^2 values than CBP-FB, unlike period 2 where the coefficients of determinations were low in both treatments. Temporal evolution of CBP temperature was better predicted in period 1 than in period 2, decreasing as pack composting days increased in period 1 and vice versa in period 2. In the case of CBP moisture, pack composting days in both periods predicted CBP moisture with R^2 values which ranged from 0.43 to 0.63. Temporal evolution of CBP moisture was similarly predicted in both periods, increasing as pack composting days increased. This can be explained by the water from the excreta left by cows on the pack. As for CBP C:N ratio, pack composting days in both periods predicted CBP C:N ratio with high R^2 values, which ranged from 0.78 to 0.97. Temporal evolution of CBP C:N ratio was similarly predicted in both treatments, decreasing as pack composting days increased. Also, this can be explained by the presence of cows on the pack and the resulting nitrogen from their excreta. In summary, among the performance variables studied, C:N ratio was where pack composting days explained the highest proportion of variance. However, RMSE values decreased from CBP C:N ratio, CBP moisture, to CBP temperature. These results suggest that temporal evolution of CBP moisture and C:N ratio, unlike temporal evolution of CBP temperature, was equal between periods, following the constant pattern of the pack at the beginning of the establishment of a CBP system, independently of differences between periods (i.e. ambient temperature). Conversely, discrepancies between periods in temporal evolution of CBP temperature suggest that it could be affected by ambient temperature, being better predicted when ambient temperature decreased. This has been confirmed by previous results of CBP performance depending on ambient temperature, where CBP temperature increased as ambient temperature increased in both periods, unlike discrepancies shown in CBP moisture and C:N ratio. Overall, temporal evolution of CBP moisture and CBP C:N ratio was accurately predicted. Although temporal evolution of CBP temperature with period 1 conditions were accurately predicted, discrepancies between periods, apparently caused by ambient temperature, meant that these equations were not useful in different conditions of period 1.

As we had collected CBP samples throughout the composting process, we conducted an additional analysis for assessing the temporal evolution per week of CBP performance, in addition to the regression analysis. The samples considered were week

0 (W 0), when the pack was still not established, for CBP moisture and CBP C:N ratio, week 2 (W 2) for CBP temperature and CBP moisture, and weeks 7 and 11 (W 7 and W11) for CBP temperature, CBP moisture and CBP C:N ratio. Pen mean of daily CBP temperature and pen mean of weekly CBP moisture and CBP C:N ratio were used for the statistical assessment. Data were statistically analyzed using the MIXED procedure of SAS (Table 5.5). Separated by period, the model contained the fixed effects of week, treatment and week × treatment interaction, and the random effect of cow. Repeated measure statement of day was used for CBP temperature. The Tukey multiple comparison test was applied to conduct mean separation across weeks and treatments.

With regard to the temporal evolution per week, all CBP performance variables presented a week × treatment interaction in both periods. As for CBP temperature, in period 1 it was higher in W 2 than in W 7 and W 11 in both treatments, and all weeks presented higher CBP temperatures in CBP-S than in CBP-FB. In period 2, CBP-S temperature was higher in W 2 than in W 7 and W 11, but W 7 was lower than W 11, whereas there were no differences among weeks in CBP-FB temperature. In any case, in all weeks CBP temperature was higher in CBP-S than in CBP-FB. These results agree with CBP temperature regressions, with a more accurate prediction of the decreased evolution of CBP temperature in period 1, and a weak prediction in period 2. In the case of CBP moisture, in both periods it increased from W 0 to W 7, whereas values were similar between W 7 and W 11 in both treatments. In W 0 and W 2, CBP-FB moisture was higher than in CBP-S. A similar temporal evolution of CBP moisture was registered in both treatments. These results agree with CBP moisture regressions, where there was a moderate prediction of temporal evolution of CBP moisture in both periods. Regarding CBP C:N ratio, in period 1 it decreased from W 0 to W 11 in both treatments, with a higher CBP C:N ratio in CBP-S than in CBP-FB in W 0. In period 2,

Table 5.5 Effect of week and bedding material on CBP performance.

Item	CBP-S[1]				CBP-FB[2]				SEM	*P*-value[3]		
	W 0	W 2	W 7	W 11	W 0	W 2	W 7	W 11		W	T	W×T
Period 1												
CBP temperature, °C	-	43.0^{a}	28.9^{c}	29.8^{c}	-	39.8^{b}	22.3^{d}	21.4^{d}	0.66	0.001	0.001	0.001
CBP moisture, %	10.0^{e}	38.6^{c}	64.5^{a}	64.3^{a}	24.3^{d}	50.3^{b}	69.1^{a}	69.6^{a}	2.41	0.001	0.001	0.014
CBP C:N ratio	312.5^{a}	-	122.0^{bc}	47.8^{d}	128.0^{b}	-	108.0^{c}	35.0^{d}	6.79	0.001	0.001	0.001
Period 2												
CBP temperature, °C	-	38.1^{a}	31.0^{c}	34.6^{b}	-	25.9^{d}	25.8^{d}	27.2^{d}	1.16	0.001	0.001	0.001
CBP moisture, %	10.5^{d}	40.8^{c}	63.7^{a}	62.9^{a}	40.0^{c}	53.4^{b}	63.3^{a}	63.1^{a}	3.13	0.001	0.001	0.001
CBP C:N ratio	263.0^{a}	-	141.5^{b}	40.5^{c}	133.0^{b}	-	105.0^{bc}	34.3^{c}	35.37	0.001	0.004	0.039

[1] CBP-S = Compost-bedded pack of sawdust treatment.

[2] CBP-FB = Compost-bedded pack of forest biomass treatment.

[3] W = week effect; T = treatment effect; W×T = Week × treatment interaction effect.

$^{a-e}$Means within a row with different superscripts differ ($P < 0.05$).

CBP C:N ratio decreased from W 0 to W 11 in CBP-S, but in CBP-FB the ratio only differed between W 0 and W 11. These results agree with CBP C:N ratio regressions, with the accurate prediction of decreased CBP C:N ratio evolution in both periods. Overall, although CBP temperature was lower in CBP-FB than in CBP-S during both periods, consequently worsening the composting process, the behavior of CBP moisture and C:N ratio did not differ between treatments in both periods, from W 7 to the end of period, in spite of the fact that CBP moisture and C:N ratio in W 0 were worse for composting in CBP-FB than in CBP-S. This means that, although forest biomass did not work as well as sawdust in terms of CBP performance, forest biomass appeared to be capable of supporting a composting process. In order to confirm this, further research with controlled initial variables is needed.

5.2.3. Effect of the composting time on compost-bedded pack (CBP) microbial counts

To assess the temporal evolution of CBP microbial counts, CBP samples were taken on W 0, when the pack was still not established, W 7 and W 11 (Table 5.6). Pen mean of weekly CBP microbial counts was used for the statistical assessment. Due to the significant period effect in statistical assessment shown in Chapter 3.2, data were differentiated by period. Data were statistically analyzed using the MIXED procedure of SAS. The model contained the fixed effects of week, treatment and week × treatment interaction, and the random effect of cow. The Tukey multiple comparison test was applied to conduct mean separation across weeks and treatments.

Total bacteria count (TBC) presented week × treatment interaction effect in both periods. In period 1, TBC were higher in W 7 and W 11 than in W 0 in both treatments.

Table 5.6 Effect of weeks and bedding materials on CBP microbial counts.

Microorganism counts, $\log_{10}$ cfu/g	**CBP-S[1]**			**CBP-FB[2]**			**SEM**	***P*-value[3]**		
	W 0	**W 7**	**W 11**	**W 0**	**W 7**	**W 11**		**W**	**T**	**W×T**
Period 1										
Total bacteria count	4.63^{c}	8.85^{a}	8.94^{a}	7.29^{b}	8.65^{a}	8.84^{a}	0.270	0.001	0.001	0.001
Total coliforms	2.00^{c}	6.29^{b}	7.29^{a}	5.20^{b}	5.98^{b}	5.27^{b}	0.513	0.001	0.245	0.001
Escherichia coli	2.00^{c}	6.03^{a}	6.00^{a}	2.00^{c}	4.70^{b}	4.38^{b}	0.440	0.001	0.000	0.027
***Klebsiella* spp.**	2.00^{c}	5.00^{ab}	5.25^{ab}	4.52^{ab}	5.39^{a}	4.35^{b}	0.402	0.001	0.003	0.001
***Streptococcus* spp.**	2.00^{c}	8.07^{a}	7.35^{b}	2.54^{c}	7.69^{ab}	7.99^{a}	0.250	0.001	0.040	0.003
Staphylococcus aureus	2.00^{c}	7.62^{ab}	7.92^{a}	2.00^{c}	6.85^{b}	6.20^{b}	0.456	0.001	0.001	0.007
***Bacillus* spp.**	3.30	6.19	5.91	4.95	7.18	6.29	0.999	0.002	0.030	0.476
Yeasts and fungi	2.00^{c}	4.78^{b}	4.86^{b}	5.68^{ab}	5.38^{ab}	5.87^{a}	0.368	0.001	0.001	0.001
Period 2										
Total bacteria count	3.36^{c}	8.65^{ab}	8.81^{ab}	7.65^{b}	8.93^{ab}	9.17^{a}	0.455	0.001	0.001	0.001
Total coliforms	2.00^{b}	5.58^{a}	5.63^{a}	5.42^{a}	5.88^{a}	5.89^{a}	0.579	0.001	0.001	0.001
Escherichia coli	2.00	5.18	5.09	2.00	5.85	5.79	0.564	0.001	0.108	0.566
***Klebsiella* spp.**	2.00^{b}	4.85^{a}	5.40^{a}	4.43^{a}	4.65^{a}	5.01^{a}	0.687	0.001	0.079	0.011
***Streptococcus* spp.**	2.00^{c}	7.58^{a}	7.26^{a}	3.15^{b}	7.66^{a}	7.34^{a}	0.314	0.001	0.011	0.030
Staphylococcus aureus	2.00^{c}	7.81^{a}	6.87^{b}	2.00^{c}	7.79^{a}	7.74^{a}	0.268	0.001	0.040	0.010
***Bacillus* spp.**	2.80^{d}	5.62^{ab}	7.00^{a}	4.89^{cd}	5.20^{bc}	7.08^{a}	0.704	0.001	0.101	0.033
Yeasts and fungi	2.50^{c}	3.89^{bc}	4.37^{ab}	5.59^{a}	5.60^{a}	5.35^{a}	0.549	0.075	0.001	0.024

[1] CBP-S = Compost-bedded pack of sawdust treatment.

[2] CBP-FB = Compost-bedded pack of forest biomass treatment.

[3] W = week effect; T = treatment effect; W×T = Week × treatment interaction effect.

[a–d] Means within a row with different superscripts differ ($P < 0.05$).

In period 2, TBC were higher in W 7 and W 11 than in W 0 in CBP-S, but in CBP-FB the difference was only detected between W 0 and W 11. Week 0 presented higher counts in CBP-FB than in CBP-S in both periods. This expected result is probably linked to the organic composition of the forest biomass. Overall, temporal evolution of TBC increased but remained steady from W 7 to the end of the period in both treatments and periods, except in CBP-FB of period 2. This could be explained by the expected increase of TBC and microorganisms counts from the beginning of a CBP because the contribution of manure to raw materials, which usually has a low microbial population, and the establishment of a balanced microbial population for composting. In our study conditions, it could be the case that CBP microbial population became balanced at W 7.

Total coliforms presented week × treatment interaction effect in both periods. Total coliform counts of CBP-S increased from W 0 to W 11 in period 1, but were higher in W 7 and W 11 than in W 0 in period 2. Total coliform counts of CBP-FB did not differ among weeks in both periods. Week 0 presented higher counts in CBP-FB than in CBP-S in both periods, whereas W 11 presented higher counts in CBP-S than in CBP-FB, but only in period 1. Black et al. (2014) reported that coliforms increased when CBP temperature increased and CBP moisture decreased. Eckelkamp et al. (2016) observed an increase in coliform counts when CBP temperature increased and CBP moisture was equal to or greater than 60%. In the present study, temporal evolution of total coliforms did not agree with the aforementioned reports. In period 1, when CBP temperature of both treatments decreased and CBP moisture of both treatments increased, being more than 60% from W 7 to W 11, total coliforms increased in CBP-S and remained steady in CBP-FB. In CBP-S of period 2, when CBP temperature decreased but increased from W 7 to W 11, and CBP moisture increased, being more than 60% from W 7 to W 11, total coliforms increased but remained steady from W 7 to W 11. And in CBP-FB of period 2, when CBP temperature remained steady, and CBP moisture increased, being more than 60% from W 7 to W 11, total coliforms remained steady. These suggested that temporal evolution of total coliforms in CBP-S appeared to grow conversely to the aforementioned reports, increasing when CBP temperature decreased, and temporal evolution of total coliforms in CBP-FB did not appear to be affected by CBP temperature. We suggested in Chapter 3.2 that in worse weather conditions and pack performance, forest biomass could control total coliform counts better than sawdust. To expand on this statement, we could speculate that forest biomass could better control temporal evolution of total coliforms than sawdust when weather conditions get worse, and vice versa. Besides this, although forest biomass presented higher total coliform counts than sawdust at the beginning of this experiment, by the end bacterial growth during the composting process was higher in sawdust than in forest biomass.

Escherichia coli counts in period 1 were higher in W 7 and W 11 than in W 0 in both treatments. Although counts in W 0 were the same in both treatments, in W 7 and W 11 they were higher in CBP-S than in CBP-FB. In period 2, *E. coli* presented a week effect, counts being higher in W 7 and W 11 than in W 0. Black et al. (2014) found that *E. coli* counts increased when ambient temperature increased. In the present study, *E. coli* increased but remained steady from W 7 to the end of the period in both treatments and periods, being not consistent with Black et al. (2014) in period 1, when ambient temperature decreased throughout the period. However, the relationship between ambient temperature and temporal evolution of *E. coli* was also unclear in period 2, when ambient temperature increased during the period, because *E. coli* remained stable for part of the period. This suggested that period conditions (i.e. weather conditions and

CBP performance) did not affect temporal evolution of *E. coli* in this study. However, period appeared to affect treatments, period 1 conditions being more conducive to the growth of *E. coli* in sawdust than in forest biomass.

Klebsiella spp. presented week × treatment interaction effect in both periods. *Klebsiella* spp. counts of CBP-S were higher in W 7 and W 11 than in W 0 in both periods. *Klebsiella* spp. counts of CBP-FB were higher in W 7 than in W 11 in period 1, but were similar between weeks in period 2. Week 0 presented higher counts in CBP-FB than in CBP-S in both periods. Eckelkamp et al. (2016) reported that *Klebsiella* spp. had no significant relationships with CBP performance. In the present study, temporal evolution of *Klebsiella* spp. performed similarly within each treatment, increasing but remaining steady from W 7 to the end of the periods in CBP-S, and remaining steady during the periods in CBP-FB. In agreement with Eckelkamp et al. (2016), these results suggested that CBP performance did not affect *Klebsiella* spp. in any treatment because temporal evolutions were similar between periods. Besides this, these results also suggested that forest biomass could better control *Klebsiella* spp. counts than sawdust, as in total coliforms.

Streptococcus spp. presented week × treatment interaction effect in both periods. *Streptococcus* spp. counts were higher in W 7 and in W 11 than in W 0 in both treatments and periods, but were also higher in W 7 than in W 11 in CBP-S of period 1. Week 0 presented higher counts in CBP-FB than in CBP-S in period 2, whereas W 11 presented higher counts in CBP-FB than in CBP-S in period 1. Black et al. (2014) reported no effect of CBP temperature on *Streptococcus* spp. counts. Conversely, Eckelkamp et al. (2016) observed a decrease in *Streptococcus* spp. counts when CBP temperature increased, but CBP moisture and C:N ratio did not affect them. In the present study, *Streptococcus* spp. increased but remained steady or decreased from W 7 to W 11 in both treatments and periods. However, the results were not consistent with Eckelkamp et al. (2016) in period 2, when CBP temperature increased from W 7 to W 11, but *Streptococcus* spp. counts did not decrease between these weeks.

Staphylococcus aureus presented week × treatment interaction effect in both periods. *S. aureus* counts were higher in W 7 and W 11 than in W 0 in both treatments and periods, but were also higher in W 7 than in W 11 in CBP-S of period 2. Week 11 presented higher counts in CBP-S than in CBP-FB in period 1, and vice versa in period 2. Black et al. (2014) observed that ambient temperature affected *Staphylococcus* spp., which exhibited some heat intolerance. Similarly, *Staphylococcus* spp. counts experienced a slight decrease with increasing CBP temperature, but with no effect due to CBP moisture Eckelkamp et al. (2016). In the present study, *S. aureus* increased but remained steady from W 7 to W 11 in both treatments and periods, except in CBP-S of period 2, where the decrease from W 7 to the end of the period matched with the increase of CBP-S temperature between these weeks, in agreement with Eckelkamp et al. (2016). Besides this, at the end of the period 1, sawdust led to a higher growth in *S. aureus* counts than forest biomass, but at the end of the period 2, forest biomass led to a higher growth in *S. aureus* counts than sawdust.

Bacillus spp. presented week and treatment effects in period 1, W 7 and W 11 counts being higher than W 0 counts, and CBP-FB counts higher than CBP-S counts. In period 2, *Bacillus* spp. counts were higher in W 7 and W 11 than in W 0 in CBP-S, and higher in W 11 than in W 0 and W 7 in CBP-FB. *Bacillus* spp. counts increased at warmer CBP temperatures and lower C:N ratios (Shane et al., 2010). Conversely, Eckelkamp et al. (2016) reported that with increasing CBP temperature, *Bacillus* spp.

counts decreased, whereas they increased with increasing C:N ratio. In the present study, *Bacillus* spp. increased but remained steady from W 7 to the end of the period in both treatments and periods, except in CBP-FB of period 2. These results did not agree with the aforementioned reports, but higher CBP-S temperatures in period 1 matched with the treatment effect of period 1, in which *Bacillus* spp. counts in CBP-S were lower than in CBP-FB, in agreement with Eckelkamp et al. (2016) but not with Shane et al. (2010).

Yeasts and fungi presented week × treatment interaction effect in both periods. Yeasts and fungi counts of CBP-S were higher in W 7 and W 11 than in W 0 in period 1, but were higher in W 11 than in W 0 in period 2. Yeasts and fungi counts of CBP-FB were similar between weeks in both periods. Week 0 of both periods, W 7 of period 2 and W 11 of period 1 presented higher counts in CBP-FB than in CBP-S. No literature was found on yeasts and fungi counts in CBP dairy farms. In the present study, yeasts and fungi increased in CBP-S but remained steady from W 7 to W 11, and remained steady in CBP-FB.

Overall, most microorganisms performed similarly, increasing until W 7 and remaining steady to the end of the period, which may be explained by the typical increase of microorganism counts at the beginning of a CBP, and the establishment of a balanced microbial population, mentioned previously. Regarding different temporal evolutions of CBP microbial counts, it seems that period 2 presented more different temporal evolutions than period 1. This suggested that period 2 conditions (i.e. weather conditions and CBP performance) challenged the establishment of a balanced microbial population. Temporal pattern was only found to be steady in CBP-FB, in both periods of total coliforms and of yeast and fungi, and in period 2 of *Klebsiella* spp. Resting temporal evolutions were all increasing in different ways, except Klebsiella of period 1 in CBP-FB, which remained stable, except for a peak in the middle of the period. These results supported the suggestion made in Chapter 3.2 that the temporal evolution of CBP microbial counts was more controlled and uniform in CBP-FB than in CBP-S, specifically in total coliforms, *Klebsiella* spp. and yeasts and fungi.

5.2.4. Economic assessment of forest biomass

In Spain, wood by-products (sawdust, shavings and wood chips) have experienced a great boom in recent years due to its usefulness as a substrate in pellet stoves or boilers. This increase has hit farmers and livestock owners financially, as they use wood by-products as bedding material for animal housing. Specifically, at the beginning of this century, sawdust quoted price fluctuated around €30 /Tn, but in 2014 the sawdust market suffered a big increase of €10 /Tn, and since then has increased around €1-2 /Tn annually, until the current price of €52 /Tn. Although the sawdust price may continue to grow until €55-58 /Tn, these values seem to be the ceiling price (Enerbio). Shavings and wood chips have undergone a similar evolution, but these by-products have a lower price than sawdust (a quoted price of around €12 /Tn), as they require less chopping (Leñas Serra). As forest biomass has not really been used as a by-product until recent years, no information about quoted price is available. Besides this, some companies process forest biomass adding other components such as essential oils or soil, which drive up the initial price.

To assess the profitability of forest biomass in CBP barns, a comparison with sawdust in CBP barns was made. The real prices of the bedding materials tested in the present study cannot be used in the economic assessment because in the case of sawdust we purchased low amounts, and no quoted market price was available for forest biomass. At the beginning of each period of the present study, pens (12.5 m^2) were filled with 30 cm of the new bedding material, obtaining a volume of 3750 L/pen. As bulk density differed between bedding materials, being 182 ± 6.3 g/L in sawdust and 240 ± 16.2 g/L in forest biomass, CBP-S and CBP-FB needed 682.5 kg/pen and 900 kg/pen of bedding material to be initially established, respectively. During the composting process, an average of 0.8 kg/m^2 of new bedding material per day was added in each pen before tilling when pen CBP moisture was greater than 60%. The average amount of new bedding material added was 7.8 kg/pen per day in CBP-S, and 7.9 kg/pen per day in CBP-FB. Regarding the initial establishment of CBP and the periodical addition of bedding material, CBP-FB required numerically higher amounts than CBP-S.

Taking as a reference the current quoted price of sawdust (€0.052 /kg) and considering the periodical addition of bedding material in our CBP-S (7.8 kg/pen), the cost was €0.4056 pen/day. Dividing this daily cost of sawdust by the periodical addition of bedding material in our CBP-FB (7.9 kg/pen), the maximum forest biomass price should be €0.051 /kg to match the economic performance of sawdust in the conditions of the present study. However, regarding the initial establishment of CBP in the present study, the initial bedding material cost in CBP-S (682.5 kg/pen x €0.052 /kg = €35.49 pen) divided by the initial bedding material used in CBP-FB (900 kg/pen) showed that the maximum forest biomass price would be €0.039 /kg. Considering the recent boom in pellet stoves and the real use of forest biomass as a substrate in them, the quoted price of €39 /Tn for forest biomass seems unrealistic. The factors that worsened the economic performance of forest biomass were its high bulk density and its high requirement of additional bedding material in the CBP, which could be explained by the high moisture content of raw forest biomass. Improvements in raw forest biomass characteristics, such as reducing moisture content, would enhance this bedding material in economic terms and the improve CBP performance and animal welfare. However, as mentioned in Chapter 3.2, we must be careful about making changes in raw forest-biomass moisture content because CBP-FB microorganism counts might be modified. Overall, although it seems that the use of forest biomass as bedding material in CBP barns may affect farm profitability if its cost is higher than that of sawdust, further studies in commercial conditions focused on economic assessment must be carried out to verify the current estimations. Besides that, factors related to milk production, and animal health and welfare, among other farm issues, should be used in this economic assessment.

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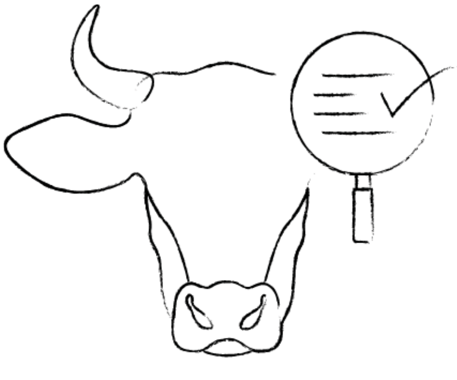

CHAPTER 6.

Final Conclusions

The farm management strategies studied in the current thesis to improve housing and feeding systems, as well as animal welfare, allow us to conclude that in our experimental conditions:

1. Regarding the specific objective in feeding system of fattening beef cattle:

- **1.1.** Increasing the content of physically effective fiber in high-concentrate diets fed to beef heifers reduces intake, affects sorting behavior, increases time spent ruminating, and reduces the number of hours under critical rumen pH related to subacute ruminal acidosis.
- **1.2.** Dietary peNDF has to be increased in cattle diets because it stimulates rumination activity, behavior related with welfare and ruminal health. However, this must be done at a level which avoids affecting animal production, because increasing dietary peNDF also reduces intake and leads to sorting against large particles.
- **1.3.** The equation of prediction for peNDF intake was accurate because the independent variables used, namely dietary peNDF proportion, dry matter intake and body weight, explained 94% of its variation.
- **1.4.** The minimum pH equation was the best to predict rumen disorders because the independent variables chosen explained 50% of their variation.
- **1.5.** Overall, in the farm management strategy in fattening beef cattle fed high-concentrate diets, the optimal level of dietary peNDF was 10.4%, because this content maintained feed intake, and improved animal welfare by limiting sorting behavior, and promoting sufficient time spent ruminating to reduce the number of hours under critical rumen pH, which minimizes the risk of subacute ruminal acidosis.

2. Regarding the specific objective in housing system of dairy cows:
    - **2.1.** The alternative bedding material used, forest biomass, did not work as well as sawdust in terms of CBP performance, because CBP temperature was lower and CBP moisture was higher with forest biomass than with sawdust. However, forest biomass appeared to be capable of supporting a composting process.
    - **2.2.** Forest biomass could be a better bedding material than sawdust with regard to reducing microbiological counts in CBP, such as total coliforms, *E. coli*, *Klebsiella* spp. and *S. aureus*, and controlling the temporal evolution of microbiological counts in CBP, such as total coliforms, *Klebsiella* spp. and yeasts and fungi.
    - **2.3.** Forest biomass did not work as well as sawdust in terms of cow comfort because time to lie down, as a measure of resting behavior, was longer with forest biomass than with sawdust.
    - **2.4.** The proportion of variance explained by ambient temperature was more accurate in CBP C:N ratio, with higher coefficients of determination, than in CBP moisture and CBP temperature, with lower coefficients.
    - **2.5.** Overall, the farm management strategy of using forest biomass as an alternative substrate to sawdust traditionally used in the resting area of compost-bedded pack barns improved pack microbial content but worsened pack performance and animal welfare. Other factors such as higher required volumes of forest biomass than sawdust and market prices of materials could have a greater economic impact on farm profitability.

3. Regarding the specific objective in the agronomic characteristics of compost-bedded pack:

- **3.1.** In both the compost-bedded pack and the composted piles, forest biomass or sawdust presented potentially valuable agronomic characteristics when it comes to organically amending the soil, as they are high in organic matter content and low in nutrients.
- **3.2.** The additional composting process of three months in the composted piles did not bring about any meaningful change to compost-bedded pack materials, and only the organic matter of forest biomass pile was stabilized.