# Effects of flavouring additives on feed intake and immune function of newly received feedlot cattle

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## Abstract

Ninety Angus × Hereford steers (259.9 ± 36.18 kg BW) were used in a 56-d experiment to assess the effects of flavouring additives on stimulating feed intake and immune function of newly received feedlot cattle. Steers were homogenously distributed by body weight (BW) into six pens (15 head/pen) and pen was randomly assigned to one of three treatments (two pens/treatment): a standard feedlot receiving diet (CT), or the same diet with a flavouring additive comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 g/kg (Lucta SA, Barcelona, Spain). Pens were equipped with a feed intake monitoring system (Growsafe Systems, Airdrie, Canada), while BW, chute behaviour, flight speed, blood samples, saliva samples were collected bi-weekly and hair samples collected at 4-week intervals during the study. Data were analyzed using a mixed-effects model accounting for repeated measures with steers as the experimental unit, except for chute behaviour, where a non-parametric one-way ANOVA was used. There were multiple treatment × time interactions ($P < 0.05$). Meal size was greater for SW than CT on wk 3 and than MX on wk 4 and 5, and it was greater for MX than CT and SW on wk 3 and 7, respectively. The daily number of visits to the feed bunk was greater for MX than CT on wk 2, for SW than MX on wk 4, and for CT than in MX and SW on wk 4, and wk 7 and 8, respectively. The eating rate was greater for SW than MX and CT on wk 4 and 5, and on wk 4 than MX, respectively. Although the effects on DMI, average daily gain and feed efficiency (FE; kg BW/kg DM) were not significant ($P > 0.1$) over the 56-d feeding period, FE and ADG were greater ($P < 0.05$) in SW and MX than CT from d 27 to 41, and ADG was lower for SW and MX steers than CT from d 15 to 28. Haptoglobin concentration in blood also showed a treatment × time interaction ($P < 0.05$), where SW steers had lower concentrations than CT on d 14. Blood fibrinogen and serum amyloid A concentrations were greater ($P < 0.01$) on d 1 than the rest of the days. Neutrophil percentage was greater ($P <0.01$) on d 56 than d 28, whereas lymphocyte percentage was lower ($P < 0.01$) on d 56 than d 28. Hair and saliva cortisol concentration were lower ($P < 0.01$) on d 56 compared to d 1 and 28, respectively. The use of flavouring additives, and mostly the one based on sweeteners, caused some positive changes in the feeding pattern and haptoglobin concentration of newly received steers. These changes, however, were not consistent over the 56-d feeding period and were not accompanied by a change in their growth performance, temperament, or in the biomarkers of stress, inflammation, or immune function. A different combination of flavouring agents, dose, or strategy to use flavouring agents (one flavour profile vs. a rotation of flavours over time) should be explored in

future research trials to further assess the efficacy of these additives in newly received feedlot cattle.

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# List of abbreviations

ACTH Adrenocorticotropic Hormone

ADF Acid detergent fiber

ADG Average Daily Gain

APP Acute-phase proteins

AVMA American Veterinary Medical Association

BCRC Beef Cattle Research Council

BRD Bovine Respiratory Disease

BW Body weight

CP Crude protein

CRH Corticotrophin-Releasing Hormone

DM Dry matter

DMI Dry matter intake

EID Electronic identification

EO Essential Oils

FE Feed efficiency

Fb Fibrinogen

Hb Haemoglobin

Hp Haptoglobin

HPA Hypothalamic–Pituitary–Adrenal

IgM Immunoglobulin M

IL-6 Interleukin 6

LS Least squares

NDF Neutral detergent fiber

NFACC National Farm Animal Care Council

NFC Non-fibrous carbohydrates

NRC National Research Council

NSAID Non-steroidal anti-inflammatory drugs

NSC Non-Structural Carbohydrates

SAA Serum amyloid A

SAM Sympathetic-Adrenal-Medullary

SEM Standard error of the mean

TMR Total mixed ration

TDN Total digestible nutrients

USDA United States Department of Agriculture

VFA Volatile fatty Acid

WBC White blood cells

# 1. General Introduction

In Canada, the transition phase from cow-calf operations to stocker or feedlot operations is always critical, as these calves are recently weaned and may have gone through different stressful husbandry practices before leaving the farm. Examples of practices may include castration and dehorning/disbudding. Marketing of calves also involves transportation which may induce stress because of the loading process, feed and water deprivation, adverse weather conditions, commingling with other cattle from unknown origin, or the processing upon arrival to the new feedlot (Schwartzkopf-Genswein et al., 2016).

The stress from the above-mentioned factors results in a decreased feed consumption upon arrival to the feedlot (Duff and Galyean, 2007), with cattle losing as much as 2% of their body weight (Preston, 2007), and with only 38.9 and 62.2% of healthy calves eating anything at all after 1 and 2 days at the feedlot, respectively (Hutcheson and Cole, 1986). The consequences of this reduced feed intake not only causes a reduced nutrient supply, but it is also compromises the permeability of the gastro-intestinal tract, which can further impair their immune function and may activate the acute phase responses (Goff, 2006; Albornoz et al., 2013; Zhang et al., 2013; Tóthová et al., 2014). Increased concentration of acute phase proteins (Cooke, 2017), cortisol (Bourguet et al., 2011), and excitable temperament (Burdick et al., 2011a) can be noticed upon feedlot entry and are indicative of stress responses. Stress has been speculated to contribute to increased susceptibility for diseases, such as bovine respiratory disease (BRD) and a poor growth performance. Metaphylactic antibiotics are widely used in beef cattle feedlots to prevent the appearance of such diseases, and hence improve their growth performance. However, the concern over the emergence of antibiotic resistance (Stevanović et al., 2018) has raised attention towards alternative solutions that may be safer for cattle and humans, and at the same time has the ability to increase productivity and make animals more efficient.

Stimulating feed intake is the main priority upon feedlot arrival (Baumont, 1996), which contributes to decreasing the incidence of disease and supporting a stronger immune function. A greater intake has been noticed by Montoro et al. (2011) in dairy calves provided with sweeteners with orange smell, and by Yang et al. (2010) in finishing cattle fed a cinnamon-flavoured diet. Blanch et al. (2016) found increased milk production in multiparous cows upon addition of

cinnamaldehyde and garlic oil to their diet. To the best of our knowledge, there are no studies on the effects of flavouring additives on feed intake of newly received feedlot steers.

## 2. Literature review

### 2.1. Beef cattle industry

The cattle population is steady or slightly declining worldwide, as it decreased by 1.4% from the year 2012 to 987.1 million in 2020 (Statista, 2020). The same trend was observed in Canada; where, in January 2020, the total cattle population was 11.2 million, which means a 3.4% reduction in comparison with the year 2016 (Statistics Canada, 2020a).

In 2018, Canada was ranked 6$^{th}$ largest beef producer (USDA, 2020a) and 12$^{th}$ biggest exporter of beef (USDA, 2020b). The production of beef cattle in Canada is unevenly distributed (Pogue et al., 2018), where 88% of the cattle population are raised in Western Canada, with Alberta and Saskatchewan accounting for 41% and 30% of all the cattle operations, respectively (Statistics Canada, 2020a; Statistics Canada, 2020b).

In Canada, in the period that goes from 2016 to 2020, the number of cattle operations has declined from 75,790 to 72,700, but the average number of cattle and calves per farm remains constant (Statistics Canada, 2020b). The same can be observed in Alberta and Saskatchewan, where the number of cattle farms decreased by 3.8% and 0.7%, respectively, with a steady number of animals per herd (Statistics Canada, 2020b). When looking at a longer period, from 2006, the reduction in the number of cattle operations is much pronounced (33%) (Jelinski et al., 2015).

In contrast with the aforementioned numbers, meat production in Canada is displaying an escalating trend to fulfill the increasing demand from consumers. The total number of slaughtering cattle has risen by 9.5% from 3.17 to 3.46 million from 2014 to 2018 (Statistics Canada, 2020c) likely because of the increasing numbers of imported live cattle during this period and declining export of live cattle. Moreover, Canada is in the 2$^{nd}$ position of increased carcass weight after USA in 2020 (Greenwood, 2021). This productivity improvement has been achieved by advancements in the use of growth promotors (implants and B-agonists), breed selection, diet, and feeding management practices.

### 2.2. Management practices of beef production in North America

Beef production practices can be divided into three main sectors based on the rearing system in North America: (1) cow-calf operations, (2) stocker or backgrounding operations, and (3) finishing feedlot operations (Endres and Schwartzkopf-Genswein, 2017). The cow-calf and

stocker operations heavily rely on forage resources, while the feedlot operations require a large quantity of grains (Galyean et al., 2011). In Canada, the function of the stocker operations are merged within the backgrounding phase (a stage right after calves are weaned that has the goal of utilizing pasture and high-forage diets to increase cattle frame size before placing them in the feedlot) at the feedlot (Sheppard et al., 2015; Pogue et al., 2018). The number of operations in each of these sectors is influenced by the different stocking density and level of intensification at each sector, with finishing feedlots having the lowest number of operations but still having the capacity for capturing all animals from cow-calf operations due to its more intensive production system (Asem-Hiablie et al., 2017).

### 2.2.1. Cow-calf operations

The beef supply chain starts at the cow-calf operations, and it will take more than two years from the moment a cow or heifer is breeding until its offspring is ready to slaughter (Comerford et al., 2013; Endres and Schwartzkopf-Genswein, 2017). The goal is to get calves from 95% of the cowherd, utilizing mostly native perennial pastures to feed the cows, and getting calves to optimal weaning weight. It is an extensive, low-input, pasture-dependent production system where calves remain with their mother until they are weaned (Endres and Schwartzkopf-Genswein, 2017) and sold to stocker operations or feedlots via direct sell or auction market. A fewer number of operations would keep a portion of their cattle and finish them on an all-forage-based diet (Asem-Hiablie et al., 2017). Cows are typically fed on native or tame pasture during summer and preserved forages (bale grazing, swath grazing, grazing on standing corn, and grazing on stockpiled forages) during winter, with mineral supplementation depending on the forage quality (Sheppard et al., 2015).

In Canada, most farmers manage the herd in a way that synchronizes the calving season with the period that goes from mid-March to May (Alemu et al., 2016). This way, the high energy demands from milking cows can be satisfied with highly nutritious emerging grass (Sheppard et al., 2015). Other producers delay the calving season to avoid adverse weather conditions and shortage of labour in early Spring, as well as to avoid fall run price of weaned calves, where price drops due to oversupply of calves in the market (Sheppard et al., 2015). The weaning weight and age range between 245-270 kg (Sheppard et al., 2015a) and 6 to 7.7 months of age, respectively

(Asem-Hiablie et al., 2017). An average of 23% of the heifers (Asem-Hiablie et al., 2017) are kept by producers for replacement purposes in the Western United States.

By the time they are weaned, calves have typically had some experience with dry feed fed at the feed bunk. Calves will also be vaccinated before weaning against infectious bovine rhinotracheitis, bovine respiratory vial syncytial virus, bovine viral diarrhea types 1 and 2, parainfluenza 3, clostridial, and *Mannheimia haemolytica* (Waldner et al., 2013; Fike et al., 2017). The vaccination process takes place two times: during branding and 7 to 14 days before any transportation event (Fike et al., 2017). According to Waldner et al. (2013), in Canada, around 85% of the cow-calf producers vaccinate their calves before leaving the pasture.

Another health management protocol is deworming, either in injectable form or pour-on, 1-2 times per year (Fike et al., 2017). In calves, this takes place at branding, or before or after weaning. Different fly control methods also exist, such as herd spraying, oil-based back rubbers, or dust bags.

### 2.2.2. Stocker operations/Backgrounding

Stocker operations, also known as backgrounding operations, continue the work done on cow-calf operations, in the sense that a forage-based diet is fed to weaned calves to build frame size before achieving the target marketable weight of around 400 kilograms (Johnson et al., 2010; Endres and Schwartzkopf-Genswein, 2017). Booster vaccinations (Fike et al., 2017) and hormone implants (Johnson et al., 2010) are given to prevent diseases and achieve additional weight gain and feed efficiency for greater profits. Stocker operations act as a bridge between cow-calf operations and feedlots, and they serve the purpose of utilizing forage resources to spread the market window for finished cattle more evenly all the year round (Galyean et al., 2011). The stocker calves typically enter the feedlot as yearlings, at 12-14 months of age (Endres and Schwartzkopf-Genswein, 2017).

### 2.2.3. Auction markets

The auction markets are an important part of the livestock industry, providing the opportunity for producers to sell and buy livestock at large. The alternative way of purchasing calves directly from a ranch is known as "ranch direct", which allow feedlots to get a more uniform but smaller lot of calves. Most of the cow-calf operations are small and geographically scattered (Alemu et al., 2016), so auction markets also provide the cow-calf producer with an increased

market visibility. This way, cattle from multiple sources can accumulate at the auction market and the feedlot operators can purchase them in large groups for backgrounding and finishing.

One of the main disadvantages of acquiring cattle via auction markets is that calves from multiple sources commingle, resulting in exposure to pathogens, stress (Schwartzkopf-Genswein et al., 2018) and an increased body weight shrink because of transportation, sometimes with multiple transportation events. Step et al. (2008) studied health and performance of recently weaned and direct-shipped steers (ranch direct) compared to steers weaned 45 days before shipping, and steers from auction market. A higher ADG upon arrival to the feedlot was reported with ranch direct compared to auction market steers. In another study of long distance transportation cattle in Canada, Flint (2013) reported that the auction market cattle were more fatigued and more likely to lie in a head supported position compared to ranch direct cattle. More recently, there has been an increase in the number of calves being sold ranch direct through video (Schwartzkopf-Genswein et al., 2018), which may be a good option in the future to reduce the number of transport events for live animals, hence reducing the risk of diseases and production loss.

### 2.2.4. Feedlot operations

Once calves are sold by either cow-calf or stocker operations, they are transported to the feedlot operation where these cattle will be introduced to a more intensive system (Alemu et al., 2016). After a step-up transition, high-grain diets are predominantly used in feedlot operations to maximize the growth rate of cattle (Samuelson et al., 2016), as well as to enhance the marbling by increasing deposition of intramuscular fat (Brand et al., 2019). Vitamins and mineral supplementation is provided with the diet to satisfy cattle physiological demands and prevent any possible nutrient deficiencies (Sheppard et al., 2015), but more on this and the nutritional management of receiving cattle will be discussed in section 2.5.3.3.

Depending on the weight of cattle and the feed available at the feedlot, the presence of a backgrounding phase aims to both acclimatize cattle to the new environment and to increase their body weight with minimal fat deposition by eating forage-based diets (Beauchemin et al., 2010). In the US, the backgrounding phase in feedlots typically lasts 90 to 110 days (Reinhardt and Thomson, 2015; Asem-Hiablie et al., 2017), while in Canada it takes around 145 d (Alemu et al., 2016), likely due to Canadian operations using more extensive backgrounding programs. Low-

weight animals may also need a longer time to adjust than heavier calves (Reinhardt and Thomson, 2015).

As the diet transitions from backgrounding to finishing, greater ADG can be observed (1 kg/d vs 1.4 kg/d) (Sheppard et al., 2015; Endres and Schwartzkopf-Genswein, 2017). The predominant cereal in the diet depends on the region and availability in the market (Sheppard et al., 2015). For example, corn is more common in backgrounding steers and heifers in Eastern Canada, as opposed to barley on the Prairies.

The finishing phase can take between 100 to 170 days (Beauchemin et al., 2010) and the average finishing age for cattle is 20 months (Asem-Hiablie et al., 2017). Some of the factors influencing the length of these periods are initial body weight and diet formulation (Alemu et al., 2016). The finishing live weight of cattle in North America is within 590-680 kg (Beauchemin et al., 2010; Reinhardt and Thomson, 2015).

### 2.3. Welfare issues associated with beef cattle production

Animal welfare plays an important role in making decisions for management practices, and different stakeholders, from slaughter plants to beef producers, are voluntary adopting verification programs to provide evidence of good animal welfare practices (Tucker et al., 2015).

#### 2.3.1. Welfare issues at cow-calf operations

Most of the standard husbandry practices known to be painful and stressful for cattle take place at cow-calf operations, among which the most important are weaning, castration, disbudding or dehorning, and branding. Besides those, other welfare issues may arise in cow-calf operations, including extreme weather conditions, insects and parasites present in the field, dystocia with poor calving assistance, scarcity of water and stressful human-animal interaction (Endres and Schwartzkopf-Genswein, 2017).

##### 2.3.1.1. Weaning

Weaning is the process by which calves are prevented to consume milk from its dam, which can be done in different ways, including abrupt weaning, fence-line weaning, or two-stage weaning (Wilson et al., 2017). Abrupt weaning consists of the calves being physically separated from the cows and, either calves or more commonly cows, transported the same day to a different location (Tucker et al., 2015). Although this is the cheapest option in terms of costs of facilities and human

labour, the accumulated stress for the calf and cow has a bigger impact in their health in the short and long-term (Grandin, 2016). A recent survey on Western Canadian cow-calf operations reported a decreasing trend of abrupt weaning from 70% in 2014 to 49% by the year 2017 (University of Saskatchewan, 2018).

Two-stage weaning, and fence-line weaning are considered more welfare-friendly than abrupt weaning. In two-stage weaning, calves are first restricted from nursing by placing a plastic anti-suckling device into the calf's nose for up to 1 week, while still keeping social contact with the dam (Haley et al., 2005). In the second week, calves are separated from the dam and the anti-suckling device is also removed. Improved behavioural signs (more time spent eating, less time walking, and fewer vocalizations) and increased BW gain in the first week has been showed in two-stage weaning compared to abrupt weaning (Haley et al., 2005; Boland et al., 2008). In the fence-line weaning method, a fence physically separates the calves from the cows, although the auditory and visual contact is still possible. There is a lower presence of behavioural signs of stress and a greater BW in fence-line weaning compared to abrupt weaning (Price, 2002; Boland et al., 2008). Despite the method used, weaning remains to be a stressful experience based on the effects detected at a physiological (e.g., plasma cortisol levels) and behavioural (e.g., vocalizations, mobility) level (Haley et al., 2005).

#### 2.3.1.2. Castration

Castration of bull calves is a routine practice in beef production to avoid unwanted breeding, to minimize aggressive behaviour towards humans and other cattle, and to improve carcass characteristics (NFACC, 2013; AVMA, 2014a; Fike et al., 2017).

Different methods of castration have been evaluated in the literature, including physical, chemical, and hormonal methods (AVMA, 2014a). In beef cattle production, castration is mostly performed using physical methods including the use of a knife or scalpel for surgical removal of the testicles, application of a rubber band at the base of the scrotum, or by crushing the spermatic cord with a Burdizzo clamp (Fike et al., 2017).

All methods of castration cause physiological (e.g. cortisol) and behavioural changes (restlessness, kicking the hind leg, head-turning, abnormal standing posture, increased recumbency, tail swishing, and reduced intake and activity) indicative of pain and stress responses (AVMA, 2014a; Wilson et al., 2017). However, the research on the different methodologies shows

that there are different pros and cons associated to each procedure. Surgical castration is associated with an intense acute pain and an increased level of plasma cortisol and haptoglobin concentration and decreased lymphocytes following castration (Stafford and Mellor, 2011; AVMA, 2014a). Possible complications include chances of hemorrhage, infection, and poor wound healing. Application of burdizzo castration (using a special clamp to crush the spermatic without surgical incision) is easier than other physical methods but it is also related to an acute increase of cortisol, severe inflammation, and more human error (Pang et al., 2009).

Compared to burdizzo castration, rubber bands cause less severe inflammation (Pang et al., 2009) but leads to a necrotic tissue due to blockage of blood supply into the scrotum (AVMA, 2014a). Rubber band castration is also associated with inflammation, swelling, hardness of the tissue around the place of band attachment, and presence of abnormal postures over a longer period of time than other physical methods of castration, suggesting the presence of chronic pain (Thüer et al., 2007), which typically remain until the testicles fall off.

There is an extensive body of publications on different pain mitigation strategies during castration. Administration of local anesthetic (e.g. lidocaine) into the spermatic cord and neck of the scrotum is effective to return the serum cortisol concentrations to baseline within 1 hour after castration (Thüer et al., 2007). However, the short action time of lidocaine meant that cortisol levels may soon return to high levels following the fade out of its effects (Fisher et al., 1996; Ting et al., 2003). Administration of anti-inflammatory drugs (e.g. meloxicam, flunixin, ketoprofen) showed a promising effect on reducing plasma acute phase proteins, cortisol concentration, and fewer pain-related behaviours (Ting et al., 2003; Marti et al., 2018; Meléndez et al., 2018). Since 2018, pain mitigation is mandatory for castrating calves older than 6 months of age (Schwartzkopf-Genswein et al., 2018).

The other factor that influences the pain suffered during castration is the age of the calves. Calves younger than 1 week of age castrated with rubber bands showed fewer behavioural signs of distress than older calves, while plasma cortisol did not differ from non-castrated calves (Mellor et al., 2002). As calves get older and the testicles develop, there is an increased risk of infection, blood loss, and death (Schwartzkopf-Genswein et al., 2012). Beef calves castrated at an earlier age before weaning had increased feed intake, ADG than calves castrated during weaning (Warnock

et al., 2012). Therefore, castration should be conducted as early as possible, ideally within the first week of age to minimize pain (Pearson et al., 2019).

#### 2.3.1.3. Disbudding and dehorning

Disbudding is the method for preventing horn growth in calves when buds are less than 5-10 mm long or before 2-3 months of age (Tucker et al., 2015). Dehorning is the process of removing the horns once they are attached to the skull. The purpose of dehorning and disbudding is to reduce the risk of injuries for cattle handlers, to reduce the risk for carcass bruises, and injuries of other cattle (Anderson, 2012). The procedures to achieve this objective can be divided into three: a) chemical application of caustic paste, b) cauterization using a hot iron, and c) surgical amputation of the horn using saws, knives, scoop, cups, tubes (Winder et al., 2016). The first two methods are applicable before the attachment of the horn bud to the skull, while the last method, depending on the tools used) can be used to remove either the horn bud or the horn once it is attached to the base of the skull. Disbudding is preferred over dehorning as it involves less tissue trauma, as the horn is still on the bud stage, and there is not physically attached to the skull (NFACC, 2013).

Chemical disbudding requires no surgical equipment and it is suitable for cattle from day 1 to 3 weeks of age (M'hamdi et al., 2013). Cauterization dehorning uses a hot iron powered by electricity, fire, batteries, or butane to burn the skin at the base of the horn from where the horn will grow (M'hamdi et al., 2013). The use of hot iron disbudding also causes less pain than amputation due to the destruction of nociceptors with the heat (AVMA, 2014b). Amputation of the horn is done by using a tube, saw, spoon, or knife to cut the horn out and prevent further growth (Anderson, 2012; M'hamdi et al., 2013). Nevertheless, whenever it is possible, polled genetics is the best option to avoid any painful procedure.

Like castration, dehorning is also a painful procedure and it can be evaluated through the measurement of physiological (cortisol, adrenaline, and nor-adrenaline concentration) and behavioural signs (e.g. head shaking, head rubbing, tail flicking, ear flicking, continuous lying and rising) (AVMA, 2014b). Using local anesthetics prevents the rise of physiological and behavioural indicators (Winder et al., 2016). The addition of an NSAID with the anesthetic agent reduced pain behaviours up to 44 hours after disbudding (Heinrich et al., 2010). In Canada, since January 2016,

pain control is mandatory with dehorning of calves after bud attachment (2 to 3 months of age; NFACC, 2013).

#### 2.3.1.4. Branding

The branding of cattle is the application of a thermal injury to the skin by placing a hot or freezing iron causing a scar and the death of pigment-producing cells to leave a permanent symbol (AVMA, 2011). Branding is considered to be the best permanent animal identification, as it makes easy to identify cattle from a distance, and it is legally accepted proof of ownership that may be required by community pastures, lending institutions, or for export.

Branding is associated with pain responses during and after the procedure, such as increased tail flicking, kicking, vocalization, falling down, and less time lying caused by sensitive tissues (Tucker et al., 2014). Both hot-iron and freeze branding are painful to ruminants (Moggy et al., 2017), and the application of NSAID does not seem to have any major effect preventing this pain (Tucker et al., 2015). The studies on hot-iron branding and freeze branding revealed that hot-iron branding produced a greater response in cortisol concentration and behavioural indicators of pain than freeze branding, while those parameters were also greater in freeze branded cattle compared to sham-branded (Schwartzkopf-Genswein et al., 1997; Schwartzkopf-Genswein et al., 1998). Alternative identification methods include ear tags, ear notches, tattoos, paint brands, or electronic identifications (Bello et al., 2020). Western Canadian cow-calf producers, however, did not find these alternatives to be an effective option (Moggy et al., 2017).

### 2.3.2. Welfare issues related to live cattle transportation

In Canada, each step of the beef cattle production system tends to occur in different regions, and multiple short or long transportation events are therefore needed (Sheppard et al., 2015). According to Schwartzkopf-Genswein et al. (2016), feeder calves are transported at least once but as many as 6 times in their life. Poor welfare outcomes related to transport include lameness, non-ambulatory cattle, and increased morbidity and mortality at the feedlot (González et al., 2012; Goldhawk et al., 2014b). These outcomes depend on multiple factors, including handling during loading, transport duration, trailer and environmental microclimate, the fitness of the animals, and the road conditions (Schwartzkopf-Genswein et al., 2016).

The loading process is the first step of the transport event, and it is one of the most stressful processes of transportation. As reported by Buckham et al. (2008) plasma cortisol concentration

increased at the onset of transportation and then declined during transportation, although it was never lower than the cortisol levels recorded 24 h before transportation. On the other hand, markers of inflammation, such as plasma fibrinogen and haptoglobin, were lower at the onset of transportation and remained lower for 48 h compared with 24 h before the transportation event. Others (Arthington et al., 2008; Marques et al., 2012; Cooke et al., 2013a) have reported increased concentration of acute phase proteins associated with cattle transportation.

Transport duration includes the waiting time after loading, driver rest periods, stationary periods of the traffic signals, and border crossing. In Canada, cattle can be transported for as long as 36 h before unloading and provision of feed and water is required. Standing for a long time during transport affects the physical condition of cattle, and when cattle are transported for longer than 30 h, the possibility for lame cases and death increases significantly (González et al., 2012a).

The microclimate inside the trailer during transport depends on as the external temperature, humidity, loading density, excretions, airflow inside the trailer, and it can affect the welfare outcomes of the transportation event (Goldhawk et al., 2014a; Schwartzkopf-Genswein and Grandin, 2014; Van Engen and Coetzee, 2018). The mortality of cattle increased sharply when the temperature was below -15$^{o}$C (L. González et al., 2012b). In cold climates, cattle try to keep themselves warm and use energy via shivering, but most of the energy is already lost due to restrictions on pre-transport feed and water (NFACC, 2013). Blocking the ventilation holes of the trailer during winter can protect animals from cold temperatures and it can reduce the incidence of dark cutters, which are considered a degraded portion of meat, appearing as a dark, dry and with grainy texture, resulting from the stress that cattle suffered during the pre-harvesting period (Goldhawk et al., 2014a; Schuetze et al., 2017). Similarly, there is a correlation between the number of non-ambulatory cases and high temperatures (González et al., 2012b).

The loss of live weight and carcass yield during transportation is both a welfare and an economic issue (Schwartzkopf-Genswein and Grandin, 2014). Weight loss during transportation is mainly associated with deprivation of feed and water, increased defecation, urination, evaporation, and respiration (Van Engen and Coetzee, 2018). A loss of more than 8% of their BW is correlated with an increased mortality during the transportation event (González et al., 2012a). Body weight before transportation is also an important predictor of their resilience, as Cernicchiaro

et al. (2012) reported that heavier calves recover more quickly from the transportation stress than lighter calves.

Finally, a link between stress and disease susceptibility has been found for different cattle diseases, such as mastitis (Sordillo and Raphael, 2013), Johne's disease (Duff and Galyean, 2007), or salmonellosis (Rostagno, 2009) in dairy cows. Incidence of BRD in cattle is also associated with the stressful conditions faced by cattle during the weaning and transportation process, exacerbated by the exchange of pathogens while commingling in trailers and/or auction markets. In this regard, Hodgson et al. (2012) noticed double mortality of weaned and transported calves when these calves were experimentally infected with BRD compared with calves that were not weaned and transported.

### 2.3.3. Welfare issues in feedlot operations

#### 2.3.3.1. Bovine Respiratory Disease (BRD)

BRD is the most important welfare and economic concern of the feedlot cattle industry, accounting for 75% of the morbidity and 50% of the mortality cases annually (Galyean et al., 1999) and causing an economic loss of $13.90 per head because of the decreased BW gain and use of antimicrobials (Snowder et al., 2006). Upon arrival on the feedlot cattle go through an initial processing that includes prophylactic vaccination, deworming, ear tag identification, and hormones implant (Schwartzkopf-Genswein et al., 2018). This handling and processing, along with the stress accumulated from the recent transport event, commingling and adaptation to the new environment is a major cause of stress that makes cattle susceptible to viral and bacterial respiratory infections (Duff and Galyean, 2007; Tucker et al., 2015; Wilson et al., 2017). Metaphylactic antimicrobials are effective at reducing the incidence of BRD and improve productivity of receiving cattle, but it is costly and may compromise the efficacy of antimicrobials in the long term due to the appearance of antibiotic resistances.

The detection of BRD is still based on visual observation of clinical signs by pen riders, which has been proved to have a modest accuracy (Thompson et al., 2006). There are studies using feeding behaviour patterns to diagnose morbidity around 4 d earlier than the clinical signs are apparent to human observers, which may increase the effectiveness of antimicrobials (Quimby et al., 2001). Wolfger et al. (2015) reported low BRD risk in cattle with longer meal durations, greater meal size, and increased meal frequency.

#### 2.3.3.2. Ruminal acidosis

Transitioning diets from high fiber (backgrounding cattle) to high grain (finishing cattle) poses a challenge to cattle and their ruminal environment (i.e., microbial activity, fermentation profile). Ruminal acidosis is one of the major digestive disorders affecting beef cattle, which can account for 10.4% of the mortalities in beef operations (USDA, 2011). In both its acute and subacute presentations, rumen acidosis has been associated with a reduced gain-to-feed ratio, growth performance and carcass weight (Castillo-Lopez et al., 2014) and an increased incidence of liver abscess and inflammatory response (Nagaraja et al., 1999; Wiese et al., 2017), with significant welfare and economic implications.

In normal circumstances, the production and absorption of organic acids from the fermentation of the ingested diet is at balance in the rumen (Nagaraja and Lechtenberg, 2007). When there is a higher percentage of highly fermentable grain in the diet, the rumen may be overwhelmed by the increased production of acids, and the accumulation of lactic and volatile fatty acids causes a reduction in the pH level, leading to acute and subacute acidosis (Aschenbach et al., 2011). The animal at this stage decreases its feed intake, resulting in low ADG and feed efficiency. The ruminal acidosis can also lead to other health issues such as liver abscesses and laminitis (Schwartzkopf-Genswein et al., 2018). Liver abscesses are reported quite frequently on finishing cattle and are also used as a measure of acidosis (Fox et al., 2009). To mitigate the effects of high-grain diets, feedlot producers are recommended to follow a "step-up" diet to gradually adapt to the high-grain diet (Aschenbach et al., 2011). There are other approaches for the prevention and treatment of acidosis in cattle, such as the inclusion of buffers in the diet, ionophores, or changing the diet formulation to ensure sufficient effective fiber is provided in the diet (Schwartzkopf-Genswein et al., 2018). These and other strategies have been reviewed by Calsamiglia et al. (2012).

#### 2.3.3.3. Lameness and weather challenges

The concern for cattle lameness has increased in the last decade, and nowadays it is considered as the second welfare issue after BRD (Schwartzkopf-Genswein et al., 2018). Characterized by the abnormal gait of the animal, lameness is mainly associated with pain and discomfort resulting from injury or infectious disease, that results in a reduced movement of the animals making it difficult to get access to feed and water (Schwartzkopf-Genswein et al., 2018).

The challenge of accurately identifying lameness, especially when it is less severe, has made it hard to investigate the prevalence of lameness in feedlots and the characteristics and risk factors associated with these cases (Stokka et al., 2001). Muddy pen conditions are associated with an increased risk of disease, injury, and lameness in feedlot cattle. For example, *Fusobacterium necrophorum* spreads from the faces of normal cattle and affects the interdigital space causing foot rot (Stokka et al., 2001). Besides this, muddy conditions in high rainfall areas is also associated with decreased feed intake and lower growth performance (Grandin, 2016). Mud creates a slippery floor condition which predisposes cattle to injury, makes locomotion difficult, and affect cattle lying behaviour (Endres and Schwartzkopf-Genswein, 2017). Besides the presence of mud, if the floor is made with a hard stepping surface (e.g. concrete), it can affect the foot conformation of cattle, making them susceptible to injuries, infections, and laminitis (Stokka et al., 2001).

Finally, heat and cold stress is another welfare concern for outdoor feedlot cattle that can affect their behaviour, physiology, productivity, and can even cause death in extreme cases (Tucker et al., 2015). The existing literature on heat stress comes mostly from the US (Grandin, 2016), where the main contributing factors are identified as lack of shade, heavy cattle weights, and coat color of cattle. Heat stress can be detected through the physiological changes such as increased body temperature, pulse and respiration rate, and assessment of panting score (Tucker et al., 2015). Black coat cattle suffer more from heat stress as a result of the increased absorption of sunlight and reduced evaporation rate (Okoruwa, 2015). On the other extreme, cattle in outdoor feedlots during winter require protection from cold temperatures in Canada.

### 2.4. Assessing beef cattle stress

As mentioned above transportation, feed deprivation, weaning, human handling, commingling, and change of environment all are factors that create different levels of stress to receiving calves. The stress response of cattle includes measurable changes at a behavioural (e.g. increased vocalization) (Haley et al., 2005) and physiological level (Lefcourt and Elsasser, 1995), which will be reviewed in the following sections.

#### 2.4.1. Cortisol

The stress response in cattle is known to trigger two different signaling axes: the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic-adrenal-medullary (SAM) axis.

The stimulation of the hypothalamus results in the release of corticotrophin-releasing hormone (CRH) and vasopressin (VP) (Mormède et al., 2007). The CRH then transmits a signal to the pituitary gland and stimulates the release of adrenocorticotropic hormone (ACTH). This hormone targets the adrenal cortex and generates glucocorticoids that are released to the blood stream. Besides the production of glucocorticoids, the stress response also results in the production of catecholamines from the adrenal medulla through the activation of the SAM (Aich et al., 2007). The overall action of corticosteroids includes maintenance of homeostasis and catabolism of fat and protein to provide energy demands (Cafe et al., 2011) which helps the animal to cope with the situation and overcome the stressors. Glucocorticoids (in faeces), and cortisol (in body fluids, saliva or hair) are used as biomarkers of stress used to evaluate cattle.

Blood and salivary cortisol can be measured shortly within minutes after the stress stimuli (Kirschbaum and Hellhammer, 1994; Bozovic et al., 2013). Cortisol enters into the saliva by diffusion process because of low molecular weight and liposolubility through the basolateral membrane of salivary gland acini (Gröschl, 2008; Bozovic et al., 2013). Increased salivary cortisol has been reported after systemic ACTH administration (Negrão et al., 2004), cow-calf separation (Hernandez et al., 2014), and milking (Negrão et al., 2004). Increased concentration of blood cortisol prior to and following transportation has been associated to human-animal interactions and microclimate conditions in the trailer (Burdick et al., 2011a). Feed restriction before and during the transportation is also linked to elevated cortisol levels (Bourguet et al., 2011).

Hair cortisol is used to determine the stress response of animals over a longer period of time (weeks or months), as cortisol is deposited, or locally produced, for as long as the hair has been growing (Macbeth et al., 2010; Burnett et al., 2014). González-de-la-Vara et al. (2011) reported an association of bovine hair cortisol and serum cortisol concentrations. However, this association is not always clear, as the cortisol from different matrices (saliva, faeces, hair, etc.) will convey information from different time periods depending on the time required for cortisol to be deposited in them (Russell et al., 2012; Stalder et al., 2012). There are differences in cortisol concentration depending on the collection site (head, neck, shoulder, hip, and tail), as different parts of the body will grow hair at different speed and of different texture (Moya et al., 2013). The response of HPA axis to repeated stressful events has produced some debate in the scientific community, with some authors reporting a sensitization (Armario et al., 2008) and others a

habituation (Koolhaas et al., 2011) to further challenges. Therefore, an exacerbated or blunted cortisol response could be expected depending on how acute or chronic its previous experiences were, respectively. For example, it would be expected to get an increased cortisol concentration in lame cattle, however, studies have reported no changes (Burnett et al., 2015) or a reduced (Jerram et al., 2020) cortisol concentration in chronic lame cattle. Increased hair cortisol concentration has been reported by Burnett et al. (2015) in multiparous cows with clinical disease (mastitis, metritis, milk fever, chronic lameness, clinical ketosis, retained placenta) compared to healthy cows. Besides this, increased hair cortisol concentration can also be found in cattle undergoing normal physiological processes that are not necessarily considered to be stressful or compromise their welfare, such as the greater cortisol concentration reported by Braun et al. (2017) throughout normal pregnancy in dairy cattle.

### 2.4.2. Cattle temperament

Animal temperament is defined as set of behavioural traits that are consistent over time and that determine how the animal will respond in any given situation (Müller and von Keyserlingk, 2006). The temperament of animals can be measured by assessing aggression, sociability, overall activity, and fearfulness in different scenarios (Réale et al., 2007). Animals with excitable temperaments are typically associated with higher levels of reactivity and increased fear responses towards humans (Sebastian et al., 2011). This group of animals have also shown to be more aggressive during handling in the chute, and this can result in a greater chance of injuring themselves, other animals, and handlers (Burdick et al., 2011b; Haskell et al., 2014).

There are many factors that influence the temperament of individual animals, such as the genetics, previous experiences, breed, age, and sex (Grandin, 1997; Burdick et al., 2011b). For example, *Bos indicus* cattle are more excitable than *Bos taurus* cattle (Haskell et al., 2014). Burrow et al. (1988) measured temperament of 18-months old bulls and heifers and reported higher temperament scores for the bulls. Aversive handling in early life can influence their overall temperament for later life (Hemsworth et al., 1996; Grandin, 1997). The rearing condition of the animals also affects their temperament. Extensive rearing system involves less human interaction, which can make animals more reactive to the intensive system as in intensive system these cattle are often observing their handlers and other personnel (Haskell et al., 2014).

Several tests have been designed to measure cattle temperament in different situations to capture their fear responses, response to handling, social separation, or social dominance, including the measurement of their flight speed, pen and chute behaviours, open field test or novel object test. When the results of those tests are based on human evaluations and scoring methods, it is considered as a subjective test. When the results are automatized with the use of technology, the test is considered to be more objective, although the results can always be subject to human, hence subjective interpretation. Subjective methods are cheaper and ease to use on farm due to lack of dependance from additional equipment (Vetters et al., 2013). However, subjective methods are more prone to human errors or biases, as the observer can hardly be blind to the treatments being evaluated. Objective measures of temperament may yield more accurate results that are free from human error and are typically recorded on a continuous scale (Hoppe et al., 2010; Vetters et al., 2013).

Flight speed measurement (also known as exit speed) is the way of measuring how fast a calf travels a specific distance upon release from a squeeze chute. Cattle with a faster exit speed to travel a specific distance are perceived as having more excitable temperaments while those with a slower exit time are perceived as being calmer. The results from this test demonstrate cattle fear from being handled by humans at the chute system. There are devices available to measure the speed of animals, comprised of two beams that are placed in front of the squeeze chute. When the animal crosses the first beam, it triggers the initiation of the timer, which will be stopped as the animal crosses the second beam. Alternatively, flight speed can also be determined by human observers scoring cattle exiting the squeeze chute as walk, trot, or canter, which correlates well with electronic measurements (Vetters et al., 2013).

Assessment of chute behaviour is a method to measure cattle response to being restrained, isolated from peers and handled by humans in a squeeze chute (Haskell et al., 2014). Grandin (2018) created a scale to measure the behaviour of cattle restrained on a squeeze in different categories: no movement, restlessness, continuous movement, or extreme struggle. Alternatively, the force or movement generated by the calf during restraining conditions can be measured by strain gauges or accelerometers mounted on the chute (Sebastian et al., 2011), and makes this measurement more objective.

The other tests to evaluate cattle temperament require further manipulation of the animals or a specific logistic structure to facilitate such evaluation, hence making them less practical to use in feedlot cattle. The open-field test, for example, determines how an animal responds to a novel situation that involves being isolated in a novel and empty pen (Burrow, 1997). This measurement does not necessarily need the presence of the human, as the animal can be recorded and the video analyzed later to measure amount of movement, location and/or vocalizations indicative of the animal sociability and exploratory traits. The novel object test involves the presence of an unfamiliar item in the pen such as a white plastic bucket, a white wooden chair, or a red inflated ball for a certain period of time (Hemsworth et al., 1996). The objective of this test is to evoke a response of fear or curiosity to determine the animal responses (Gibbons et al., 2009). Finally, social dominance is a component of social behaviour that contributes to the establishment of social hierarchy to access resources by a herd. Dominance relationships within a herd are important as they may determine whether certain animals have the ability to access resources such as feed and water, thus contributing to the overall productivity and health of the animal. Cattle agonistic behaviours can be measured using a video camera or by direct observation of a herd. (Langbein and Puppe, 2004).

Differing perceptions and responses to a stressful stimuli in calm versus temperamental cattle may hence influence the innate immune response required for prevention or recovery from common feedlot diseases such as BRD Complex (Hulbert et al., 2011). Moreover, temperamental cattle may be less prone to display noticeable symptoms of illness when they are reacting to human handling, which makes them harder to identify when sick and thus will not get the treatment they require in a prompt and timely manner (Hulbert et al., 2011).

Excitable animals have higher baseline levels of cortisol and exhibit higher levels of plasma cortisol in response to handling, which can further result in more complicated conditions on the health of animals (Cafe et al., 2011; Haskell et al., 2014), as chronic periods of high cortisol levels have been found to have an immunosuppressive effect (Griebel et al., 2014). As consequence, Reinhart et al. (2009) reported an increased mortality rate in more excitable steers when compared to their calmer counterparts. The results of a study conducted by Hulbert et al. (2011) showed that temperamental cattle had higher levels of neutrophils but fewer functional neutrophils than calm animals. Animals with excitable temperament are also associated with lower immunocompetence

(Bruno et al., 2016), heightened acute phase response (Francisco et al., 2012; Cooke, 2017), and decreased reproductive performance (Cooke, 2017).

Finally, cattle with excitable temperament profiles have been found to spend less time at the feed bunk, had a lower feed consumption, and a decreased growth rate (MacKay et al., 2013; Llonch et al., 2018; Neave et al., 2018). The relationships between temperament and growth performance could be due to changes in the feed intake pattern, different time allocation for ingestive behaviours (Bruno et al., 2018) or the allocation of energy from growth towards coping mechanisms in excitable cattle (Haskell et al., 2014). The body weight at weaning of Angus × Herford cattle reared in an extensive system was lower in cattle with excitable temperament compared with calm calves (Francisco et al., 2012). Nkrumah et al. (2007) reported feedlot cattle with the excitable temperament to have a decreased performance and carcass quality in contrast with calmer cohorts, while Bruno et al. (2016) showed that feedlot receiving calves with low exit speeds had higher DMI and greater daily gain during the experimental period.

### 2.4.3. Feeding behaviour

The study of cattle feeding patterns has been improved with the introduction of automatic monitoring system. There are several technologies that are currently used to record the activity of animals at the feed bunk, such as Insentec® (Hokofarm group, Marknesse, Netherlands) or AniTrace (Santa Clara, CA) to monitor feeding and watering behaviour (Richeson et al., 2018). The Growsafe System (Airdrie, Alberta, Canada) is one of those technologies that can monitor the feeding behaviour of individual animals within a group or pen (Sowell et al., 1998; DeVries et al., 2003). The system was validated and originally used for recording feeding behaviour data linked the health of feedlot steers and dairy cows (Sowell et al., 1998; Schwartzkopf-Genswein et al., 1999; Basarab et al., 2003). The Growsafe system consists of electronic identification (EID) ear tags, a passive transponder, an antenna, a capacitor, a reader panel, load cells, and a personal computer for data collection. The feed bunks are equipped with load cells to measure the disappearance of feed after each visit of the animal to the feed bunk. The antenna is incorporated into a rubber mat, lining the interior surface of the feed bunk, which records the presence of an animal into the feed bunk via detection of EID ear tags when it is within the range of the antenna (50 cm). The reader panel sends then the data to the personal computer linked with Growsafe

(McAllister et al., 2011), recording the number of visits to the feed bunk, time spent on the feed bunk, and the disappearance of the amount of feed (Weary et al., 2009).

Cattle typically eat in bouts, as they concentrate multiple visits to the feed bunk in a short period of time, which is known as "meal". There are two different ways to pool visits to the GrowSafe bunk into meals in beef cattle. On the one hand, the method initially proposed by Yeates et al. (2001) and later developed by González et al. (2008b) uses the distribution of total non-feeding intervals to determine, at an individual level, the minimum time required to consider two periods of eating activity as separate meal events. On the other hand, an inter-individual meal criterion of 300 seconds, initially reported by Sowell et al. (1998) and later validated by Gibb and McAllister (1999) has been also used in the literature to determine whether two feeding events are part or not of the same meal (i.e. if the interval between two visits to the feed bunk is shorter than 300 s, then they are considered to be part of the same meal, otherwise they belong to two different meal events). Once the number of visits to the feed bunk and meals are known, other feeding behaviour parameters can be calculated, such as meal duration (in minutes), numbers of meals per day or meal size (in kg DM).

Cattle feeding behaviour can be influenced by multiple factors, including availability and quality of the feed provided, weather conditions, or social interactions. Previous studies have shown changes in cattle feeding behaviour due to morbidity, as a way to increase their resting time to hold energy for heat production and an elevated immune response (Hart, 1988; Wingfield, 2003). Richeson et al. (2018) showed that morbid calves reduced their feeding frequency and duration, while increased water consumption and drinking frequency compared to healthy animals. In a 32 days study of 108 receiving steers using the Growsafe System, Sowell et al. (1999) found light-weight morbid calves spent 52% less time eating than healthy steers. In addition, in a 227-d trial, Schwartkopf-Genswein et al. (2005) reported less time spent at the feed bunk (81 minutes per day) by steers diagnosed with BRD compared to healthy steers (104 minutes per day). Sowell et al. (1998) also reported healthy steers spending 30% more time on the feed bunk than morbid steers.

Therefore, the use of automated feeding behaviour monitoring techniques can be helpful to identify sick animals in early stages prior to physical symptoms appear. Quimby et al. (2001) reported that morbid animals with BRD can be detected using Growsafe 3 to 4 days earlier than

pen checkers via conventional observation. Moya et al. (2015) showed that the use of pattern recognition techniques applied to feeding behaviour could be used to predict the health status of feedlot heifers with a 79.5% accuracy up to 7 d before the diagnosis was made via visual observation of clinical signs. In addition, Wolfger et al. (2015) linked reduced risk for BRD with increased meal frequency, meal duration, and meal size.

### 2.4.4. Acute phase response

The acute phase response is the systemic reaction of the body to infection, tissue damage, inflammation, stress, or injuries (Ceciliani et al., 2012; Tothova et al., 2014). This response includes the production and release of a vast number of inflammatory mediators and cytokines (Tothova et al., 2014), which initiate a cascade of events including fever, anorexia, leukocytosis, and the release of non-structured proteins to the blood or biological fluids, collectively known as acute-phase proteins (APP) (Ceciliani et al., 2012).

The APPs are produced from hepatocytes and from peripheral tissue, and their function include defending the host from pathogenesis, scavenging of toxic substances, balancing homeostasis, and regulation of different stages of inflammation (Petersen et al., 2004; Tothova et al., 2014). Besides these, cytokines can also elevate the production of adrenocorticotrophic hormone and glucocorticoids, and they activate the blood coagulation system (Tothova et al., 2014). Some of the most important parameters that can be used to assess the inflammation and the acute phase response in cattle under different scenarios will be reviewed below.

#### 2.4.4.1 Interleukin 6

The response to an inflammatory process includes the release of pro-and anti-inflammatory cytokines (Jaffer et al., 2010). The main pro-inflammatory cytokines are TNFα, IL-1, IL-6, IL-8, and macrophage inflammatory protein-1α. IL-6 is produced by several cell types, such as T cells, B cells, monocytes, fibroblasts, osteoblasts, keratinocytes, mesangial cells, endothelial cells, and some tumor cells (Kishimoto, 1989). This cytokine increases the release of acute-phase proteins, induces leukocytosis, fever, and stimulates chemotaxis (Veldhuis et al., 1995; Jaffer et al., 2010). Besides the acute phase response, it also contributes to chronic inflammation through mononuclear cell accumulation at the site of injury (Atreya et al., 2000). Mihara et al. (2001) and Takagi et al. (1998) studied patients who had severe sepsis (body's extreme response towards infection) and found a correlation between increased concentration of IL-6 and mortality. It also activates the

HPA axis which can result in increased adreno-corticotrophin releasing hormone (ACTH) and cortisol levels in plasma (Tsigos et al., 1997). All this suggests that increased levels of IL-6 can be found in inflammatory processes, injuries, and in morbid animals.

#### 2.4.4.2. Serum amyloid A

Serum amyloid A (SAA) is one of the major acute phase proteins, produced primarily by the liver after the induction from pro-inflammatory cytokines, such as IL-6 and TNFα (Uhlar and Whitehead, 1999). It scavenges the cholesterol from dying cells and thus prevents the accumulation of atherosclerotic plaques (preventing to build up fat, cholesterol, or other substances in the artery walls and preventing the narrowing of the artery walls) (Manley et al., 2006). SAA acts as a chemoattractant and mediates the migration, adhesion, infiltration of monocytes and neutrophils (Badolato et al., 1994). The bovine SAA has antibacterial activity against gram-positive and gram-negative (Molenaar et al., 2009). Plasma SAA concentration increases more with acute rather than chronic inflammation (Horadagoda et al., 1999). Increased SAA have been recorded in cattle with various diseases, such as mild and moderate mastitis (Eckersall et al., 2001), bovine respiratory syncytial virus (Horadagoda et al., 1999), and severe sole ulcers and white line abscesses (Kujala et al., 2010).

#### 2.4.4.3. Haptoglobin

Haptoglobin (Hp) is another important acute-phase protein that acts as the principal scavenger of free haemoglobin (Hb) in the blood (Ceciliani et al., 2012) which reduces the levels of free iron in the blood and reduces the oxidative damage caused by free Hb (Buhman et al., 2000; Ceciliani et al., 2012). This reduction of free iron also works as bacteriostatic, as bacteria needs iron to grow. Haptoglobin reduces the inflammatory reaction after forming the Hp-Hb complex with macrophages, which upregulates anti-inflammatory mediators such as IL-10 (Philippidis et al., 2004; Schaer et al., 2006). Measuring Hp levels can aid in the diagnosis of several inflammatory processes, such as enteritis, peritonitis, pneumonia, endocarditis, and mild and moderate mastitis (Eckersall et al., 2001). Cattle exhibited increased Hp levels during viral diseases such as foot and mouth disease (Höfner et al., 1994), bovine viral diarrhea (Ulutas et al., 2011), or when challenged with classical swine fever virus (Kim et al., 2009).

Both SAA and Hp were also found as a sensitive markers of bovine respiratory syncytial virus infection in calves (Orro et al., 2011). Acute phase proteins can also be elevated during

stressful procedures, such as, transportation and weaning. Serum concentrations of Hp and SAA increased significantly in transported cattle after 48 h (Lomborg et al., 2008), and 3 and 5 d post-weaning (Kim et al., 2011). Serum Hp concentration in calves on arrival at the feedlot, however, was not associated with the overall performance and had limited value in targeting treatment for calves (Holland et al., 2011).

#### 2.4.4.4. Fibrinogen

Fibrinogen (Fb) is another APP, and a precursor of fibrin, which is the final product of the conversion of a clot to an insoluble fibrin form (Davalos and Akassoglou, 2012). The fibrinogen levels have been found to increase two to three-fold in inflammatory conditions and in response to infections (Hirvonen and Pyörälä, 1998; Medcalf, 2007). The increased levels of fibrinogen are also associated with atherosclerosis, acute thrombosis, and increased risk of cardiovascular diseases in humans (Eidelman and Hennekens, 2003). Elevated concentrations of Hp and Fb were reported by Tóthová et al. (2012) in calves having multi-systemic diseases where more than one organ was affected. For example, higher concentrations of Hp and Fb were reported by Gånheim et al. (2007) in calves that had both diarrhea and respiratory diseases compared with only diarrhea or respiratory diseases. In addition, calves that were suffering from diarrhea and had high concentrations of Hp, Fb, and SAA had these concentrations reduced with the initiation of treatment (Jawor and Stefaniak, 2011).

#### 2.4.4.5. Immunoglobulin M

Serum immunoglobulins are an important indication of the immune reaction against pathogenic microorganisms. Most of the research on immunoglobulins (Ig) is limited to the passive transfer of Ig in dairy calves. When providing commercial yeast culture to 30 dairy cows and heifers to evaluate the effects on immune transfer, Wafa (2017) found that calves fed the highest dose had greater serum concentrations of IgM. Xiaoping et al. (2020) offered a blend of cinnamaldehyde, eugenol, and capsicum oleoresin to ewes, and they found an increased concentration of IgM compared with control ewes. Boushehri et al. (2021), however, studied the effect of antioxidants and prebiotics on colostrum immunoglobulins of dairy cows, and they found no effect on IgG, IgM, and IgA.

#### 2.4.4.6. White blood cells

White blood cells or leukocytes (neutrophil, eosinophil, and basophil granulocytes, monocytes, and lymphocytes) are produced by the bone marrow lymphoid tissue (Roland et al., 2014). The percentage of each type of white blood cell changes as cattle grows older, with newborn calves having more granulocytes than lymphocytes (Jones and Allison, 2007; Wood and Quiroz-Rocha, 2010), whereas the percentage of lymphocytes becomes dominant and reaches up to 80% of the total white blood cells within 3 months of age (Roland et al., 2014). After that period, the percentage of lymphocytes declines but it remains the dominant leukocyte in the circulation with neutrophil being the second dominant leukocytes at a 1:2 neutrophils-to-lymphocytes ratio (Jones and Allison, 2007; Wood and Quiroz-Rocha, 2010). During an acute inflammatory process (1-2 d), the number of neutrophils in cattle decreases because of a small bone reserve for granulocytes (Roland et al., 2014). However, an increased percentage of neutrophils (neutrophilia) can be found in chronic inflammation processes (mastitis, metritis, peritonitis) (Gründer, 2006; Tornquist and Rigas, 2010) or during stressful processes associated with the abomasal displacement, ketosis, digestive upset, and dystocia (Zadnik, 2003; Webb and Latimer, 2011; Yıldız et al., 2011). Kraft and Dürr (2005) and Webb and Latimer (2011) also reported neutrophilia in non-infective inflammations (traumatic injuries, necrosis, infarction, burns, thrombosis) and neoplasia, intoxication, endocrine disorders, hemorrhage, and hemolysis.

Corticosteroids affect the number of leukocytes and neutrophils in the peripheral circulation (Burdick et al., 2011b; Hulbert et al., 2011). A key effect of glucocorticoids is the increase in segmented neutrophils (Burton et al., 2005; Hulbert et al., 2011), stimulating the release of mature neutrophils into the blood stream resulting in neutrophilia (Hulbert et al., 2011). O'Loughlin et al. (2011) found that stress caused during weaning in beef calves resulted in neutrophilia, increased neutrophil-to-lymphocyte ratio, and reduced lymphocyte number.

Lymphocytosis (increased lymphocyte) has been reported by Kraft and Dürr (2005) and Webb and Latimer (2011) during the healing phase of infectious diseases, neoplasia and hyperadrenocorticism. Gründer (2006) reported increased lymphocytes with the infection of bovine leukopenia virus. However, lymphocytopenia happened with viral, bacterial infection, during acute stress, or immune suppression (Kraft and Dürr, 2005; Jones and Allison, 2007).

## 2.5. Possible solutions to welfare issues in beef cattle production

### 2.5.1. Preconditioning

Preconditioning is the procedure of preparing calves so they have a reduced stress response to the changes associated with the transportation from cow-calf to feedlot operations (BCRC, 2014; Wilson et al., 2017). This can include weaning and vaccinating calves 30 to 45 days prior to marketing, while introducing processed feedstuff, feed bunks, and waterer (Wilson et al., 2017). It may also include castration and dehorning performed as early as possible with the purpose of reducing disease susceptibility following the marketing of calves, and make them ready to adjust to various stressors (Wilson et al., 2017; Ward et al., 2019).

Research on different preconditioning protocols shows that it reduces the incidence of health problems, decreases the treatment costs of sick calves, increases DMI, and increases weight gain compared to non-preconditioned calves (Ward et al., 2019). Step et al. (2008) reported less morbidity on calves weaned 45 days before transportation with (9.5%) or without vaccination (5.9%) compared to abrupt weaning with immediately transported calves (35.1%). In a 2-year study with 96 steers, Arthington et al. (2008) studied the performance of calves weaned with different strategies: A) weaned and shipped on the same day, B) allowed to feed on concentrate before weaning and shipping, C) weaned and feed on concentrate before shipment, and D) early-weaned (70 to 90 days prior to shipping). The overall ADG and feed efficiency were greater in D than in A steer (36.7% and 29.4%, respectively). Dry matter intake was greater in B, C, and D calves compared to A calves.

Some cow-calf producers are getting benefits from the additional value of preconditioned calves (Ward et al., 2019). According to the report from USDA (2018), preconditioned calves were sold US$35-50 more expensive than non-preconditioned calves. However, in order to implement a preconditioning program, additional labour and logistics are needed for adapting the calves to processed feeds, feed bunk, and waterer. This along with the increased risks that have to be assumed by the cow-calf producer, in terms of weight loss and possible health issues arising during the pre-conditioning program, makes this approach not worthy for some producers (Ward et al., 2019).

### 2.5.2. Rest-stop during transportation

The provision of a rest-stop during long transportation events has the goal of providing cattle with access to feed and water and a space to rest and lay down (Meléndez et al., 2021). The transport regulation of Canada (Federal Health of Animals Regulations: Part XII, Transport of Animals) has recently been changed to reduce the maximum transit time from 48 to 36 h before a mandatory rest-stop, which has also been extended from 5 to 8 h (AgCanada, 2019). Marti et al. (2017) studied the effect of rest stops of 4 different lengths during a 20-h journey on recently weaned calves under Canadian transport conditions, and they showed that cattle provided with a 5-h resting period had less cortisol concentration than cattle provided no rest or a 10-h or 15-h resting period, but the rest stop of different lengths (0, 5, 10, or 15 h) had no impact on the BW loss caused by the transportation event. Another study (Meléndez et al., 2020) found no effects of resting periods of 0, 4, 8, or 12 h on transport-related stress. More studies on the quality, quantity and duration of these rest stops are needed to determine the effects on cattle welfare and hence inform regulations on live cattle transportation.

### 2.5.3. Adequate management of receiving feedlot cattle

The proper management of newly received feedlot cattle, in terms of animal handling, housing and nutrition, plays a key role in minimizing their stress level and facilitating the transition to a new diet and environment (Samuelson et al., 2016). Starting with the proper handling of calves while they are unloaded from truck, when passing by cattle without using painful tools such as electric prods or sticks, and a relaxed posture of caregivers encourage cattle to move to their new pens and allow cattle to build up trust and confidence (Noffsinger et al., 2015). Beyond this acclimation period, low-stress cattle handling techniques will also help to shape cattle behaviour during the rest of the feeding period, to make calves less reactive and scared to human handling in the future (Noffsinger et al., 2015; Grandin, 2019), and hence more resilient to stress-induced diseases (e.g. BRD). Besides animal handling, Noffsinger et al. (2015b) and Reinhardt and Thomson (2015) also talked about three important needs for newly received cattle, which will be discussed below: rest, water and feed.

#### 2.5.3.1.Housing conditions

The housing conditions of feedlot cattle (indoor loose barn housing vs dry yard feedlots) depends on the stage of production, but also the climate, region, and logistics of the farm (Park et

al., 2020). Indoor housing showed increased performance (increased final live weight, ADG), but their greater stocking density causes cattle to spend more time standing and showing more agnostic behaviours (Braghieri et al., 2011; Tuomisto et al., 2015). Space allowances per cattle in the feedlot is critically important as it is linked to their performance. Increasing the space allowance from 3 to 4.5 $m^2$ per animal have been shown to increase live weight gain (Fisher et al., 1997; Keane et al., 2017), and feed efficiency (Hickey et al., 2003) of indoor beef cattle. These cattle also showed more lying time and positive social behaviours (licking, sniffing, and nuzzling) (Fisher et al., 1997), with fewer head-resting and abnormal behaviours than when provided 1.5 $m^2$ per head (Ruis-Heutinck et al., 2000).

Regarding outdoor pens, regular cleaning of pens, provision of bedding material, building slope away from the feed bunk, proper drainage, and reducing the stocking density can reduce the accumulation of mud in the feedlot, which is important to prevent injuries, infections, and laminitis (NFACC, 2013; Grandin, 2016). Providing shade and/or sprinklers to the animals has been proved to reduce the panting scores, and improve ADG and DMI in situations when heat stress may be a problem (Grandin, 2016). In Canada and the northern states of the US, cattle in outdoor feedlots during winter require protection from cold temperatures. Using bedding, installation of windbreak fences and changes of feeding management (especially in backgrounding cattle, where dietary energy density can be increased as the energy requirement increases due to heat loss) can improve the daily gain of these cattle.

#### 2.5.3.2.Provision of water

Providing fresh water is a key component that affects feed intake, growth performance, and physiology of feedlot cattle (Morgan, 2011). The amount of water cattle normally drink is 3 to 4 times their DMI, but this can increase under high ambient temperatures due to water loss from evaporation (Reinhardt and Thomson, 2015; Wagner and Engle, 2021). Receiving cattle that have been transported may have been off feed and water for long periods of time, which may induce a state of dehydration that further influences their reluctance to eat upon arrival to the feedlot (Hogan et al., 2007). Providing fresh clean water shortly after entering the feedlot can increase cattle feed intake by more than 1% compared to providing feed alone (Preston, 2007). Brew et al. (2011) used fifty 250-kg BW calves to evaluate how access to water caused an increased ADG compared to

those for which water was restricted for 7 days after arrival to the feedlot, suggesting an increased feed intake with water consumption.

#### 2.5.3.3.Nutritional management

A balanced diet with sufficient amount of protein, energy, trace minerals, and vitamins is required for newly received cattle to satisfy their nutritional needs and help them expedite the adaptation process. In a total mixed ration (TMR), the roughage should be around 40% of the diet (DM basis) of a good quality grass, hay, or silage (Reinhardt and Thomson, 2015; Samuelson et al., 2016). The targeted concentration of protein should be 14-16% of the diet DM (Reinhardt and Thomson, 2015) to compensate the low consumption of feed due to the stress-responses upon arrival (Samuelson et al., 2016). The specific composition of the final diet will depend on market price and availability, but Lofgreen et al. (1981) showed that a mix of hay and grain in the receiving diet increased feed intake by 39% and ADG by 83% compared to providing hay alone on the first week of the receiving period.

A speedy transition to high-grain diets may pose a risk for cattle developing acidosis. Reinhardt and Thomson (2015) suggested that the first day, receiving calves should be offered 0.25% to 0.5% of their BW in feed (DM basis). Once calves are able to consume this amount, the amount of feed offered should be increased by 0.5% of BW per day or every second day until they eat the receiving diet at 2.5 to 3.0% of their BW. It is at this moment when the authors suggest that calves can be managed and stepped up conventionally to a high-grain diet.

Vitamins and minerals are important supplements that help cattle with the normal function of the body. Vitamin A is important for the vision, reproduction, and immune function while vitamin D is important for calcium and, phosphorus metabolism, and bone development. Vitamin E, an antioxidant, interacts with selenium and is involved in the immune function of cattle. Comparing a low dose (2,200 IU/kg) vs a high dose (11,000 IU/kg) of vitamin A during the receiving period, Zinn et al. (1996) noticed a decreased ADG (20%) and feed efficiency (19%) in calves receiving the high dose. National Research Council (2016) recommends a dose of 2,200 IU/kg for feedlot cattle. Similarly, Duff and Galyean (2007) indicated that a daily dose of vitamin E greater than 1000 IU/animal reduced BRD morbidity. Others (Carter et al., 2005) found that supplementing receiving calves with a high dose of vitamin E (2000 IU/animal) had no effects on their performance and health status for the first 28 days at the feedlot. In this regard, the latest

revision of the National Research Council (NRC, 2016) on beef cattle nutrient requirements recommended a dose of 400 to 500 IU/animal daily during the receiving period for feedlot cattle.

Minerals are divided into 2 groups based on the amount supplemented in the diet: macro- and micro-minerals. Among the macro-minerals, calcium, phosphorus, and potassium are particularly important to include in the receiving diet. Calcium is added to the diet for maintenance and growth, and functions as blood clotting, secretion, and activation of certain hormones and enzymes (NRC, 2016). Samuelson et al. (2016) suggested that the level of calcium should be greater in the receiving than in finishing cattle (0.88% vs 0.73% of the diet on DM basis, respectively). Phosphorus is also an important part of bone formation, but it is also required for the growth and cellular metabolism of ruminal microorganisms (NRC, 2016). Geisert et al. (2010) showed that increasing the dose of phosphorus from 0.1 to 0.38% of the diet (DM basis) improved intake and ADG of finishing cattle. Finally, potassium is a critical electrolyte in the immune and metabolic function, and it should be provided upon arrival to the feedlot as it has been lost due to transport dehydration (Reinhardt and Thomson, 2015). Increasing the level of potassium from 0.9 to 1.3% on post-transit calves significantly increased their DMI and ADG by 27% and 14% the first 28 days at the feedlot (Hutcheson et al., 1984). The percentage of potassium recommended by Reinhardt and Thomson (2015) is 1.2 to 1.4% of diet (DM basis).

Regarding the micro-minerals (or trace minerals), a survey on consulting nutritionists made by Samuelson et al. (2016) showed that they recommend to add trace minerals to the receiving diet at a greater or equal amount than in finishing diets. Trace minerals, such as copper and zinc are mobilized from the body storages during the stress resulting from transportation, and they should be replenished via supplementation in the diet. Felix et al. (2012) offered a concentration of copper at 0, 100, and 200 mg/kg of diet to 84 crossbreed yearling steers and heifers, but they did not find any differences in intake or ADG. Similarly, providing a dose of 30, 45, 60, and 90 mg zinc/kg of diet did not cause any change in cattle intake or performance (Caldera et al., 2017). However, Chirase et al. (1991) offered different concentrations of zinc (31 vs 90 ppm) to feedlot steers challenged with infectious bovine rhinotracheitis virus, and found that DMI was reduced 50 vs 15% due to the challenge in the 31 vs the 90 ppm group, respectively, suggesting using an increased level of zinc in the receiving diet.

### 2.5.4. Feed additives

Feed additives are non-nutrient compounds, typically a mixture of molecules or organisms, that can act stimulating nutrient ingestion, absorption or assimilation, as well as improving cattle growth and health (Watts et al., 2020).

#### 2.5.4.1. Ionophores

Among all the feed additives in the market, ionophores have showed the most consistent effect on positively altering rumen fermentation. Ionophores such as lasalocid, monensin, salinomycin, laidlomycin, narasin are commonly used in livestock diets. Monensin is the predominant ionophore used in feedlot diets in North America (Samuelson et al., 2016). Monensin reduces the growth of gram-positive bacteria resulting in increased concentration of propionate and decreased acetate concentration (Duffield et al., 2012; Ogunade et al., 2018; Marques and Cooke, 2021). Increasing production of propionate is associated with the decrease of lactate and methane production. Increased propionate production was reported in forage-fed beef steers with the supplementation of monensin at 200 mg/d (Ellis et al., 2012; Ogunade et al., 2018). Adding monensin in vitro resulted in improved propionate production by inhibiting the growth of *Butyrivibrio* genus, which are important acetate and butyrate producers (Callaway et al., 1999).

Feed efficiency of ruminants supplied with ionophores increases because of the combined effect reducing feed intake plus the optimization of the diet fermentation in the rumen, with the increase propionate production, low methane production, and reduction of ruminal proteolysis and ammonia concentration in the rumen (McGuffey et al., 2001). As a result, there will be an optimal energy efficiency as well as an increased ruminal peptide and amino acid concentration for microbial protein growth, enhancing the flux of protein into the small intestine to increase muscle production (Marques and Cooke, 2021). A meta-analysis of the use of monensin in feedlot cattle showed an increased daily gain by 2.5%, decreased DMI by 3.1%, and increased feed efficiency by 1.3% (Duffield et al., 2012). A monensin-containing diet offered by Goodrich et al. (1984) to 16,000 feedlot cattle increased BW gain by 1.6% and reduced feed consumption by 6.4% compared to a non-supplemented diet. Therefore, using monensin in the feedlot cattle is an effective option to improve performance of cattle and increase economic profit.

#### 2.5.4.2. Essential oils

Essential oils (EO) are secondary metabolites from plant extracts (Benchaar et al., 2008), a mixture of low-molecular weight terpenes, alcohols, aldehydes, and/or ketones that can be obtained from leaves, seeds, stem, roots, and bark of plants (Nehme et al., 2021). The use of these additives in cattle feed have increased in recent years as it is considered a more natural alternative to increase productivity and reduce dietary issues than ionophores (Stevanović et al., 2018). Essential oils that are used in livestock and poultry include oregano, cinnamon, garlic, thyme, black pepper, lavender, or peppermint oil (Mucha and Witkowska, 2021). A variety of properties have been attributed to these additives, including flavouring, antimicrobial, antioxidants, food preservatives and growth promoters (Mucha and Witkowska, 2021; Nehme et al., 2021). Additionally, in ruminants, essential oils have been studied for their effects on rumen fermentation, such as increased VFA, or defaunation (removal of protozoa), which are associated with increased performance of ruminants.

The antibacterial activity of EO has been thoroughly described in the literature (Benchaar et al., 2008; Stevanović et al., 2018; Nehme et al., 2021), and it has been used to reduce pathogenic or methanogenic bacteria (Günal et al., 2017; Stevanović et al., 2018). The growth promoting feature of EO has been mainly associated with the improved palatability of the feed and, in monogastric, the stimulation of the secretion of digestive fluids, stabilizing intestinal microbes, reduced inflammation, and overall improving the morphology of the intestine (Stevanović et al., 2018). The studies on the effects of EO on the rumen have reported increases, decreases, or no changes on the VFA production. In a culture study by Busquet et al. (2005), a low dose of cinnamaldehyde (31.2 mg/L) and a high dose of garlic oil (312 mg/L) showed an increased propionate proportion with decreased acetate concentration. However, Castillejos et al. (2006) found a decreased proportion of propionate with the supplementation of eugenol at 500 mg/L. Increased propionate proportion in the rumen is viewed as a favorable outcome in terms of maintaining healthy fermentation profile, but it has been linked with a reduction of the voluntary feed intake (Oba and Allen, 2003).

Inflammation of the digestive tract, a physiological response to fasting or acidogenic diets, facilitates the permeability of mucosal epithelial cells and the release pro-inflammatory cytokines (Nehme et al., 2021). Essential oils can directly or indirectly interact with inflammatory

parameters: directly by blocking the synthesis and secretion of inflammatory mediators (histamine, pro-inflammatory cytokines, prostaglandin, etc.), and indirectly, by the acceleration of leukocytes recruitment (Xu et al., 2020). Essential oils and polyphenols have showed a reduction of serum amyloid A, haptoglobin, lipopolysaccharide-binding protein, and circulating neutrophils in ruminants (Ceciliani et al., 2012; Nardi et al., 2014).

However, the effects of EO in the rumen are highly variable depending on the chemical composition of each EO, the dose used, and the methodology of the study (Nehme et al., 2021). To reduce the variability of their effects and increase the accuracy and efficacy at the targeted region of the digestive tract, EO manufacturers are making these products encapsulated to increase the stability, bioactivity, and bioavailability of these feed additives (Stevanović et al., 2018; Mucha and Witkowska, 2021).

#### 2.5.4.3. Yeast products

Probiotics are active microorganisms (e.g. yeast) with an appropriate number of viable cells which growth in the host may cause a benefit in their health, BW gain, milk production, or alteration of rumen fermentation profile (Markowiak and Śliżewska, 2018). In the case of yeast, it is a unicellular eukaryotic organism that has been associated with the alteration of rumen fermentation profile to increase ADG, improve meat quality in beef, and milk production in dairy cows, as it will be discussed below.

Feed additives based on active yeast are basing its effects on yeast competing for sugar with the lactic acid-producing bacteria (*Steptococcus bovis* and *Lactobacillus*) in the rumen. Other yeast-based products (essential nutrients derived from non-active yeast cells) are also commercialized as prebiotics to promote the growth of beneficial lactate-utilizing bacteria in the rumen (e.g. *Megasphera elsdenii*) (Amin and Mao, 2021) , which can potentially reduce the accumulation of lactate in the rumen preventing rumen acidosis (Amin and Mao, 2021). Pinloche et al. (2013) reported an increased growth of *Megasphera elsdenii* following yeast supplementation to cows fed a high-grain diet. A meta-analysis made by Desnoyers et al. (2009) on yeast supplementation reported an overall increase of rumen pH, but there are several studies reporting no difference on rumen pH after yeast supplementation (Xiao et al., 2016; Amin and Mao, 2021). The differences in the effect of yeast may be due to the different products used, dosages, number of viable cells, type of feed, or feeding strategy (Amin and Mao, 2021).

Moreover, the effect of some of these yeast products preventing abrupt falls in rumen pH is associated with improved DMI, increased BW gain and improved feed efficiency (Schwartzkopf-Genswein et al., 2004). Yeast inclusion in the feed of Holstein bull calves promoted feed intake, growth rate, and rumen development, as reported by Adams et al. (2008). Inclusion of yeast products in the diet of newly received feedlot cattle have showed potential effects on DMI and body weight. Finck et al. (2014) reported an increased DMI with yeast supplementation at 5 g/d on 184 newly weaned crossbred steers. Newly received beef heifers also showed a linear increase of body weight, G:F, and ADG for the initial 2 weeks when receiving a dose of yeast at 2.5 g/d (Young et al., 2017). Ponce et al. (2012) also reported an increased DMI with a commercial yeast culture at 1.8 g/d per heifer for 35 days. These authors also reported an increased ADG for the initial 2 weeks of the experiment and a trend for an increased ADG for the whole feeding period.

#### 2.5.4.4. Flavouring additives

The presence of smell and taste in the food generates oro-sensorial signals that have been associated with satiety effects (Blundell et al., 1994), as it provides the brainstem with information that is processed along with other signals from hepatic and gastric efferent nerves on circulating metabolites (glucose), hormones, and gastric distention (Allen, 1996; Allen et al., 2009; Allen, 2014), all involved in decision making process around satiety that takes place at higher cortical regions (hypothalamus, amygdala, parahippocampal gyrus, orbitofrontal cortex, thalamus, insula) (Lasschuijt et al., 2021).

The hedonic value associated to a particular feed can override normal satiety responses and feed intake regulatory mechanisms leading to, for example, excessive intake (Baumont et al., 1990). Such hedonic value is largely modulated by the taste, although in grazing animals there are other senses also associated with it, such as the senses of sight, smell and touch (Ginane et al., 2011), which influence their selection of plants and parts to forage. In ruminants, taste buds are responsible for the taste, containing a large number of taste receptor cells and three types of papillae: circumvallate, foliate, and fungiform (Ginane et al., 2011) that are sensitive to different tastes. Cattle, sheep, and goats have lingual receptors for all five basic tastes (sweet, bitter, salty, sour, and umami).

#### 2.5.4.4.1. Sweeteners

The sweet taste has a positive hedonic value in cattle and goats (Ginane et al., 2011). Sixteen week-old dairy calves preferred to consume more water when supplemented with sucrose, fructose, glucose, galactose, glycine, and saccharin; and they showed a preference for sucrose, likely due to the sweet taste (Hellekant et al., 1994). Sheep, however, did not show the same preference for sweet taste, and increasing the dose of sweeteners actually resulted in a reduced intake or rejection (Ginane et al., 2011). Further investigation is required to know the differences of sweet taste response in sheep in contrast with other ruminant species.

Sucram, a sodium saccharin-based additive (Pancosma S.A., Geneva, Switzerland), was offered by McMeniman et al. (2006) at 0 or 200 mg/kg on DM basis to 200 newly received feedlot steers for 56 days. The author found a 5.4% greater intake with sucram during the last 4 weeks of the experiment, although the overall DMI did not differ. There was also a trend for an increased ADG (7%) for the supplemented steers. The supplemented steers also showed an increased G:F only during the first 4 weeks of the study. The same authors also measured BRD morbidity in those steers but found no effect of sucram supplementation. Later, 180 of these receiving steers were allotted for the finishing period with or without supplementation of sucram, and the authors noticed an interaction between the receiving and finishing period for DMI, where the greatest intake was found in the group supplemented in both phases. There was also a trend for increased ADG during the finishing period in the group that always received sucram. The same sweetener was studied by Ponce et al. (2014) for 56 days and provided at 0, 100, 200, and 300 g sucram/ton of feed (as DM) to 173 newly received feedlot steers. The authors did not find any interaction of treatment and time on growth performance or G: F. The DMI increased cubically with the increased supplementation of sucram, and the greatest DMI was found with the 200 g-dose of sucram. The author also measured the effect of sucram on the incidence of respiratory diseases, and they did not find any difference on the morbidity rate with the inclusion of sucram.

#### 2.5.4.4.2. Sour, salty, bitter and umami flavours

Sour taste is associated with the presence of acids in the feed. Adding low amounts of lactic and acetic acid in silage of sheep increased their intake (Bachmanov and Beauchamp, 2007). Sheep also showed preference for hay supplemented with low doses of malonic and butyric acid

(Gherardi and Black, 1991). On the contrary, cattle reduced silage intake with the presence of high doses of volatile fatty acids (Os et al., 1995), suggesting that ruminants may consume more sour taste feed when the intensity of the flavour is low. However, the specific attraction towards sour taste is not clear, as acids are naturally present in the plants, and different organic acids are also in ensiled forages, making it difficult to differentiate between what may be positive hedonic response towards the sour taste, or rather learnt response based on the positive post-ingestive feedback caused by these organic acids at the digestive tract (Ginane et al., 2011).

Similarly, the preference for salty taste can be influenced by bodily needs of sodium and minerals (Bachmanov and Beauchamp, 2007). Weaned calves lacking salt during the milk feeding stage showed a greater preference for sodium salt compared to calves that did have access to salt over the same period (Bell and Sly, 1979), likely to compensate sodium deficiencies carried over. Beyond the bodily needs for sodium, the salty flavour per se is also capable of stimulating feed intake, as shown by the 26% increase in pelleted hay consumption when supplemented with salt in sham-fed sheep (esophageal fistulated animals, so food was diverted into a bag attached to a fistula instead of the rumen after ingestion) (Grovum and Chapman, 1988).

With low-intensity bitter flavours, ruminants showed indifference on feed intake, but with increasing intensities the hedonic value seems to decrease (Ginane et al., 2011). However, among ruminants, goats showed increased tolerance to bitter flavour, likely because goats tend to naturally graze on dicotyledonous plant (among many) which produce bitter-tasting secondary compounds (Hofmann, 1989; Ginane et al., 2011). Bitter flavour is generally associated with the presence of toxins, and it is common to see ruminants reducing the intake or rejecting bitter-tasting feed (Favreau et al., 2010). For example, sheep did not show a preference for bitter-tasting hay even when associated with positive post-ingestive effects, while they did show an increased preference for umami-tasting hay with the same post-ingestive association (Favreau et al., 2010).

Umami taste is generally associated with the presence of protein in the feed, and it has the most positive hedonic value compared to other taste flavours (Ginane et al., 2011). Umami flavour has been proved to stimulate feed intake in animals (Grovum and Chapman, 1988; Favreau et al., 2010), and the addition of monosodium glutamate in the starter diet of dairy calves increased their intake during 5 weeks after weaning (Waldern and van Dyk, 1971).

Using these flavours, alone or combined, has shown promising effects in changing animal growth performance directly or indirectly. Directly, by increasing the feed consumption or acceptability of novel feeds (Villalba et al., 2011); and indirectly, by modifying rumen function (Blanch et al., 2016). Villalba et al. (2011) used thirty five 2-months old lambs provided with different combination of flavours (plain ration of alfalfa and barley, with sweet, umami, or bitter) and monotonous diet (only one of the 4 flavours). Lambs fed the flavoured diet consumed more feed and grew faster than the group fed monotonous diet. To study the effect of flavouring additives on dairy calves starter intake, Montoro et al. (2011) provided a sweetener combined with orange smell in the milk replacer of 22 Holstein calves since 7 d before weaning to 14 d after weaning. Calves offered the flavoured starter tended to have an increased starter consumption and greater ADG following weaning than non-flavoured calves.

Cinnamaldehyde, the main active component of cinnamon oil, available in the bark of cinnamon trees, is used in animals as a feed additive (Yang et al., 2010). This additive has been showed to inhibit the growth of *Prevotella spp* (bacteria involved in deamination) (Ferme et al., 2004), to increase propionate ratio in the rumen (Busquet et al., 2005), and subsequently improves gain and feed efficiency. Yang et al. (2010) provided cinnamaldehyde to 70 yearling steers at 0 (control), 400, 800, or 1600 mg/steer/day for 112 days to see the effects on intake, gain, carcass characteristics, and blood metabolites. They found an increased intake (13%) and a tendency for an increased ADG of the steers provided with cinnamaldehyde on the first 28 days. However, there were no effects during the whole experimental period on ADG, feed efficiency, and carcass traits. A mixture of cinnamaldehyde and garlic oil showed a reduced methane (68%), acetate (8%), and ammonia-N concentration (9%) with increased production of propionate (18%) and greater volatile fatty acid concentration in 24 hours *in vitro* incubations indicating a potential greater energy supply to the animal (Blanch et al., 2016). Increasing the dose resulted in decreased VFA suggesting negative effects on rumen fermentation. The *in vivo* part of this study demonstrated increased milk production in multiparous cows (additional 3 l/d) after 15 days of adaptation to the supplemented diet than control multiparous cows without affecting milk composition. The additional milk production may be associated with increased energy supply (less loss of methane and increased production of propionate) in the multiparous cows while the primiparous cows may use this for their growth.

## 2.6. Conclusions

The feedlot receiving period is one of the most critical phases of the beef cattle production system as cattle face multiple stressors due to the transport and change of environment and diet. This transition causes a decrease in feed consumption, as cattle spends less time at the feed bunk, and makes them more susceptible to diseases, which has an impact on cattle welfare and lowers their productivity. Preconditioning is one of the recommended strategies to reduce the stress and increase dry matter intake and weight gain of receiving cattle. However, the need for specific logistics and more human labour, and the way the beef industry is structured, prevents this approach from being used by most producers. Using metaphylactic antibiotics reduces the incidence of stress-related diseases, but pressure from consumers to reduce the use of antimicrobials because of the awareness of the emergence of resistances may prevent this strategy to be used in the future. Complementary or alternative approaches include the use of feed additives that aim to modify the rumen fermentation profile to reduce inefficiencies and increase the profitability. A different approach, that has been proven effective in other species, is the use of additives that change the hedonic properties of the diet, in order to stimulate feed intake. The use of flavouring agents have been shown to increase feed consumption in dairy calves and finishing beef cattle. To the best of our knowledge, there are no studies assessing the use of flavouring additives on receiving beef cattle to stimulate intake and reduce the negative effects of the stress upon arrival to the feedlot.

## 2.7. Objectives of the thesis

The objective of the current thesis is to evaluate the effects of flavouring additives on stimulating intake and immune function of the newly received feedlot cattle. More specifically:

1. To evaluate the effects of flavouring additives on feeding behaviour, growth performance, feed efficiency, and temperament of the newly received feedlot steers (Chapter 3).
2. To assess the effects of flavouring additives on acute phase proteins, inflammatory mediators, and cortisol concentration of the newly received feedlot steers (Chapter 4).

The hypothesis for these studies is that using of flavouring additives in newly received feedlot steers will cause a hedonic response that will have an impact that may be visible on an optimized feeding pattern, greater feed intake and efficiency, greater daily gain, calmer temperaments, improved immune function, lower cortisol levels, or a reduced inflammatory response.

# 3. Effect of flavouring additives on feeding behaviour, production performance, and temperament of newly received feedlot cattle

## 3.1. Introduction

Receiving feedlot cattle may be exposed to several stressful events that affect their feed intake and productivity, including recent weaning at the farm of origin, transportation, commingling, processing and change of environment at the receiving farm (Tucker et al., 2015; Endres and Schwartzkopf-Genswein, 2017). Quickly getting newly arrived feedlot cattle on feed is key factor leading to optimal cattle health and performance, and for reducing death loss and use of antimicrobials (Duff and Galyean, 2007).

Sensory properties of the feed affect its hedonic response in calves, thus stimulating or depressing intake (Baumont, 1996). Previous studies have shown that dairy calves increased the starter intake with sweeteners and orange flavour (Montoro et al., 2011), while finishing beef cattle increased their DMI with the addition of cinnamon flavouring (Yang et al., 2010). However, there is no research on the use of flavoured feeds on receiving feedlot cattle, and the possible impact on productivity and stress-mediated diseases.

The study of farm animal behaviour has advanced in the last decades up to the point of becoming an important tool to evaluate animal health (Hine et al., 2019) and welfare (Mattiello et al., 2019). Calves are variable in their availability to cope with new diets (Brown et al., 2006), and the study of their feeding pattern would allow us to have a better understanding of individual needs within a herd, as opposed to managing all cattle based on group-average information (Moya et al., 2011, 2014).

Moreover, the individual response and resilience to a stressful situation can be further scrutinized by analyzing cattle temperament (Fell et al., 1999). Cattle with excitable temperament profiles had higher baseline cortisol levels and plasma cortisol concentration (Cafe et al., 2011; Haskell et al., 2014), and they have been found to spend less time at the feed bunk, had a lower feed consumption, and a decreased growth rate (Llonch et al., 2018; MacKay et al., 2013; Neave et al., 2018). Previous research (Ulrich-Lai et al., 2007) showed that providing rats with palatable food (sweet taste) during stress decreased the physiological and behavioural effects of the stress

stimulus and increased the intake of food. Therefore, an opportunity exists for flavouring additives to attenuate the stress responses, as seen in their feed intake and temperament of cattle.

The objective of this trial is to assess the effects of feed flavours to stimulate intake of newly received feedlot cattle, and the consequences on growth performance, feeding behaviour and cattle temperament. We hypothesize that flavouring agents known to increase the hedonic response in ruminants will promote feed intake, with a positive impact in calves' feeding behaviour, performance and temperament.

### 3.2. Materials and methods

This work was approved by the University of Saskatchewan's Animal Research Ethics Board (animal use protocol: 20190123) and adhered to the Canadian Council on Animal Care guidelines for humane animal use(Ottawa, ON, Canada). The experimental phase of this study was conducted from November 2019 to January 2020 at the Livestock Forage and Excellence Centre (LFCE) of the University of Saskatchewan (Saskatoon, Canada).

#### 3.2.1. Animals used

Ninety Angus × Hereford steers (mean ± SD: 253.7 ± 36.7 kg BW) from a single source ( direct purchase from producer and transported at the same time) were transported into the research facilities and processed as per feedlot standards, including deworming with ivermectin (Boehringer Ingelheim, Burlington, Ontario, Canada), hormone implants (Zeranol, Merck Animal Health, Millsboro, United States), and vaccinations (7/SOMUBAC AND BOVI-SHIELD GOLD, Zoetis Canada Inc., Quebec, Canada). Each steer received two ear tags: one for farm identification, and another one an ear transponder to monitor feed bunk attendance (Growsafe, Airdrie , Alberta, Canada), as described below.

Each pen had one water trough where fresh water was supplied during the whole experimental period. Feed was offered twice daily in the GrowSafe bunks (GrowSafe System, Airdrie, AB, Canada) to ensure ad libitum access. Slick bunk management was practiced by evaluating the contents of the feed bunks before each feeding to minimize orts while avoiding cattle behaviours indicative of being hungry at feeding. Every pen was equipped with 3 individual Growsafe bunks (0.91 m × 0.53 m × 0.38 m), each supported by two load cells that continuously measured the amount of weight disappearance (Mendes et al., 2011). Cereal straw was used as the

bedding material and distributed opposite to the feed bunk. The back of the pens had windbreak panels to provide steers with protection from the weather.

### 3.2.2. Experimental design, diet and treatments

The duration of the study was 56 d. Steers were weighed on 2 consecutive days at the start of the study with the weight on d 1 used to stratify steers into 6 pens (15 x 20 m; 15 steers per pen, 20 $m^2$/steer). Three treatments were then assigned to those pens (2 pens per treatment, the treatments were on sequential orders such as: control followed by sweeteners and mix of basic tastes, respectively, ensuring that two adjacent pens did not have the same treatment): a standard feedlot receiving diet (control, **CT**; Table 3.1); or the same diet with flavouring additives comprised of either sweeteners (Luctarom Feedlot, **SW**) or a mix of basic tastes (Luctarom Feedlot Mix, **MX**) at 1 kg/Tn (both provided by Lucta SA, Barcelona, Spain). Diet composition was designed to meet or exceed NRC recommendations (NASEM, 2016). The larger than expected variation in CP and Ca: P values between treatments (Table 3.1.) was likely the result of an unforeseen sampling and/or mixing error, as the ingredients were added to the feed truck as originally designed, which should yield more similar chemical compositions. One animal died (MX treatment) on d 4 of the study because of a *Histophilus* infection, leaving one pen with 14 steers instead of 15 (total steers came down to 89). Other health issues were evenly distributed across pens and treatments (2 CT, 1 SW and 3 MX steers had to be treated for bovine respiratory disease).

### 3.2.3. Feed and residues DM determination

A sample of the feed offered to each pen was collected weekly from the feed bunk within 30 min of being fed in the morning to the steers. Each sample was then divided in two previously weighed paper bags and dried in the oven for 72 h at 55°C. The DM content of each sample was then calculated after subtracting the weight of paper bag and averaging the value of the two subsamples corresponding to the same pen. Samples of residual feed were collected weekly from each pen before feed was offered in the morning and processed as described for the fresh feed samples.

### 3.2.4. Sampling protocol

Throughout the whole experimental period the GrowSafe System (Airdrie, AB, Canada) was used for monitoring the individual feed intake and feeding behaviour of the steers. Every pen

was equipped with 3 individual Growsafe bunks. Scale readings (kg) from each feed bunk were transmitted every second to the computer system using data acquisition and analysis software (DAQ 3000, Growsafe System Ltd.) (Schwartzkopf-Genswein et al., 2011a; Brand et al., 2019). An antenna in each feed bunk turned on the radio frequency ear transponders when they were within a range of 50 cm, so the animal feeding could be identified.

**Table 3.1.** Composition (as DM basis) and chemical analysis of the adaptation (d 1 to 7 of the study) and experimental (d 8 to 56) diets provided to the steers throughout the 56-d experiment.

| | Adaptation period[1] | | | Experimental period | | |
|---|---|---|---|---|---|---|
| | Treatments[2] | | | Treatments[2] | | |
| | CT | SW | MX | CT | SW | MX |
| Ingredients, % DM | | | | | | |
| Barley silage | 13.7 | 13.7 | 13.7 | 50 | 50 | 50 |
| Grass hay | 49.5 | 49.5 | 49.5 | 0 | 0 | 0 |
| Dry rolled barley | 26.34 | 26.34 | 26.34 | 39.54 | 39.54 | 39.54 |
| Canola meal | 10.35 | 10.25 | 10.25 | 10.35 | 10.25 | 10.25 |
| Salt, white | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 |
| JJM micro (shelter valley) | 0.025 | 0.025 | 0.025 | 0.025 | 0.025 | 0.025 |
| Rumensin/Vitamins[3] | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 |
| Sweeteners (SW)[4] | 0 | 0.1 | 0 | 0 | 0.1 | 0 |
| Mix of basic tastes (MX)[4] | 0 | 0 | 0.1 | 0 | 0 | 0.1 |
| Chemical composition[5]: | | | | | | |
| CP, % DM | 13.2 | 16.7 | 15.1 | 15.34±0.92 | 14.67±0.78 | 15.26±0.91 |
| ADF, % DM | 28.9 | 28.6 | 29.0 | 18.59±1.22 | 21.16±2.13 | 18.34±2.08 |
| Ca:P ratio | 1.56 | 1.83 | 1.78 | 0.61±0.01 | 0.63±0.03 | 0.64±0.03 |
| NSC, % DM | 25.9 | 23.0 | 24.9 | 37.43±2.13 | 32.59±3.01 | 37.46±4.92 |
| Crude Fat, % DM | 1.4 | 1.5 | 1.5 | 2.12±0.24 | 2.38±0.38 | 2.12±0.16 |
| NFC, % DM | 33.3 | 32.2 | 34.0 | 44.76±2.23 | 40.91±3.24 | 44.89±3.86 |
| TDN, % of DM | 60.4 | 59.4 | 60.4 | 68.96±1.59 | 67.33±1.83 | 68.93±2.45 |

[1] All data expressed as least square means. Data collected from one sample collected on d 7, hence the lack of standard deviation.
[2] Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/t (both provided by Lucta SA, Barcelona, Spain).
[3] Target Vitamin A, IU/day: 25,200; Target Vitamin D, IU/d: 2,520; Target Vitamin E, IU/d:158.
[4] Products provided by Lucta SA (Barcelona, Spain), and included in the standard receiving diet in exchange for 0.1% DM of canola meal.
[5] Based on the nutrition profile of TMR of each treatment using wet chemistry analysis by a commercial laboratory (Cumberland Valley Analytical Services, PA, USA) and shown as means±SD.

Therefore, the data captured by the Growsafe system included information on all the visits to the different feed bunks in each pen, including individual number of visits to each feed bunk, length of each visit, time spent with their head down into the bunk, and feed consumed during each visit. From these data, multiple parameters (detailed below) were calculated on a daily basis, and then summarized weekly.

A visit to the feed bunk was recorded when one steer was detected with their head inside one of the Growsafe feed bunks (with or without head down). The average number of times each steer visited the feed bunks per day was calculated as the daily frequency of visits. The average length of these visits was calculated daily as the total time spent inside the feed bunk divided by number of visits, and the same was done to calculate the average amount of time spent with the head down into the feeding bunk per visit and day. Average feed consumption (in grams of DM) per visit was calculated by dividing the consumption of feed per day by number of visits that day.

Visits to the feed bunk were pooled into meals using the 300 s criteria described by Schwartzkopf-Genswein et al. (2002). Briefly, if two consecutive visits from the same animal were recorded in less than 300 s (irrespective of which of the 3 feed bunks in the pen were used), they were considered to be part of the same meal. Consequently, consecutive visits separated by more than 300 s were considered to be part of different meals. Daily meal frequency was then calculated as the number of meals an animal would take in a day. Meal duration and size were calculated as the average length that each steer needed to complete a meal and the average amount of feed ingested in each meal. The number of visits needed to complete a meal is referred as visits per meal, while the time spent by a steer with its head down at the feed bunk during each meal was calculated as head down per meal. Finally, eating rate was calculated as the average feed consumption per minute, while the head down eating rate was calculated as the daily intake divided by the time spent at the feed bunk with the head down.

Feeding events where the consumption of a single visit was less than 30 grams (as fed basis) were discarded based on the assumption that either steers were displaced from the feed bunk or had no intention of consuming feed. A Growsafe system data validation was performed daily throughout the experimental period. Briefly, the GrowSafe system has an internal audit system that calculates the daily assigned feed disappearance (AFD) for each node by dividing the total daily feed delivered to each tub by the daily sum of individual animal feed intakes as attributed by

the GrowSafe System for a specific tub (Durunna et al., 2011). In order for the data from each feed bunk to be included for data analysis, the AFD had to be above 95%, otherwise the data from that feed bunk, and consequently from the whole pen for that day, was excluded from all analyses. Over this 8-week trial, the data from the GrowSafe bunks ended up being valid for a minimum of 5 days per week. However, low AFD values were recorded throughout the study due to connectivity issues, power outages, low temperatures or heavy snowstorms.

The function of load cells was also verified weekly as described by Brand et al. (2019). Briefly, after emptying the feed bunk and calibrated by placing a 20 kg weight in each feed bunk and confirming that the system recorded the correct input at correct time. Antennas were checked according to Schwartzkopf-Genswein et al. (2011b) by placing an unassigned transponder within the read range for 15 sec. Finally, data were checked in the system to identify that all transponders were being detected at feed bunk as described by Brand et al. (2019).

### 3.2.5. Daily intake, BW, FE, and ADG

The fresh matter consumed daily by steers each day was converted into dry matter intake based on the weekly dry matter analysis. Individual body weight was collected prior to feeding while the steers were in the squeeze chute with suspended load cells on day 0, 1, 14, 28, 42, 56 and 57. Initial and final BW was determined from the average weight of first and last 2 consecutive days, respectively. Body weight gain was calculated by subtracting initial from final BW within each 2-week period. Average daily gain was calculated from dividing BW gain by the number of days of the feeding period. Feed efficiency was then calculated as the BW gain during every weighing period (d 14, 28, 42 and 56) divided by the amount of feed ingested during that period.

### 3.2.6. Cattle temperament

A flight speed device (Polaris Multi-Event Timer, FarmTek Inc., Wylie, TX) was used to measure the speed of the steers as they exit the squeeze chute on d 1, 14, 28, 42, and 56 of the experiment. The device is comprised of 4 light beams mounted on tripods and a timer. The first set of two light beams were placed against one another, each in one side of the alley in which steers were released from the squeeze, and at 1.3 m from the chute. This was considered to be the best distance to capture the speed of the animal upon release but still providing enough clearance space between the beams and the chute for the head of the animal not to interfere with the light beans

while they were still restrained in the chute. The second set of two light beams were placed in the same way, but 3.6 m from the squeeze, to provide a total of 2.3 m of run length in between sets of light beams. The distance needed to successfully evaluate cattle exit speed typically ranges between 1.5 to 2.5 m (King et al., 2006; Arthington et al., 2008; Vetters et al., 2013; Kelsey and Colpoys, 2018), and mostly depends on the type of chute and the presence of elements (fences, gates, direct exposure to Sun light) that need to be avoided for the devices to work. As each steer was released from the squeeze chute and walked along the alley in front of the chute, the first two beams were crossed, triggering the timer to start. When the steer crossed the second set of beams, the timer stopped. The time registered in the timer was recorded as seconds to cross the 2.3 meters distance between the two sets of light beams, and then converted to meters per second.

An accelerometer device (HOBO Pendant G) was affixed to the squeeze chute with vet wrap and used for measuring chute reactivity of steers. The device recorded the overall movement of the chute at a rate of 20 recordings per second. From the data collected, we used the information collected from 10 to 40 seconds after closing the headgate, while the steers were restrained for sampling, to calculate total movement, average movement per unit of time, highest and lowest acceleration captured, and standard deviation of the overall movement.

### 3.2.7. Statistical analysis

All statistical analyses were completed using SAS OnDemand for academics® (SAS institute Inc., SAS Campus Drive, Cary, North Carolina, USA). For feeding behaviour, parameters were checked for normal distribution using the Proc Univariate of SAS. Normality was assessed based on the histogram for data distribution, tests for normality, the curve of normal quantiles, and plot of residuals. Most of the parameters (head down time per day, number of visits per meal, head down time per meal, consumed feed per meal, duration per visit, head down time per visit, consumed feed per visit, eating rate) needed to be log transformed ($log_{10}$) to achieve normal distribution. Then, mixed model analysis of SAS was used including treatment, week, and the interaction of both as fixed effects, accounting for repeated measures. Pen was treated as random effect and steers were considered the subject. An unstructured covariance parameter was applied after comparing Akaike Information Criterion and Bayesian Information Criterion as the best fit statistics for the tested variables. Tukey method was used for multiple comparison test. The same model was used for feed efficiency, average daily gain, and flight speed. For chute behaviours,

normality could not be achieved, so a non-parametric one-way ANOVA of SAS was used, where treatment was applied as a classification variable, and a pairwise multiple comparison was used to compare treatment groups (n=30 steers per treatment) within each day. All data presented in tables and figures are LS means and SEM obtained from the SAS output, except for those variables which raw data had to be transformed to achieve normal distribution, in which case the LS means and SEM were reverse transformed for a better interpretation of the presented results. Significance level was always considered as $P < 0.05$.

### 3.3. Results

#### 3.3.1. Growth performance and feed efficiency

There was a treatment × day interaction ($P < 0.01$, Table 3.2), where the average daily gain (ADG) was greater in SW steers on d 1-14 and d 29-42 than MX and CT steers, respectively (Figure 3.1); MX steers had a greater ADG on d 29-42 compared with CT steers; and CT steers had a greater ADG on d 15-28 compared to SW and MX steers.

**Table 3.2**. Effects of flavouring additives on the growth performance and feed efficiency of newly received feedlot steers (n=90) during the 56-days feeding period.

| | Treatments[1] | | | | P-value | | |
|---|---|---|---|---|---|---|---|
| | CT | SW | MX | SEM[2] | Treatment | Day | Treat*day |
| Feed efficiency, kg BW/kg DMI | 0.17 | 0.18 | 0.17 | 0.02 | 0.66 | <0.01 | 0.03 |
| ADG, kg/d | 1.11 | 1.17 | 1.13 | 0.11 | 0.69 | <0.01 | <0.01 |
| Initial BW[4], kg | 259.00 | 260.95 | 259.80 | 6.72 | 0.98 | -[3] | - |
| Final BW[5], kg | 321.51 | 326.66 | 323.53 | 8.87 | 0.92 | - | - |

[1]Data expressed as least square means. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/Tn (both provided by Lucta SA, Barcelona, Spain).

[2] Standard error of the mean.

[3] The effects of day and interaction of treatment and day were not assessed for initial and final BW.

[4] Initial and final BW of the steers measured at two consecutive days

There was a treatment × day interaction ($P < 0.05$), where the feed efficiency was greater in MX and SW steers compared to CT on d 29-42 (Figure 3.2). Considering the whole 56-d feeding period, we found no significant effects of flavouring additives on the ADG and feed efficiency, or in the initial and final body weight (Table 3.2).

### 3.3.2. Feeding behaviour

There was a treatment × week interaction ($P < 0.01$, Table 3.3), where DMI was higher for SW steers at week 6 compared to rest of the weeks (Figure 3.3). We also found a treatment × week interaction ($P < 0.01$), where the total time spent at the feed bunk was greater in MX than SW steers on week 4 and 5; it was greater in SW steers than CT and MX steers on week 7; and it was greater in CT than SW steers on week 5 than SW steers (Figure 3.4).

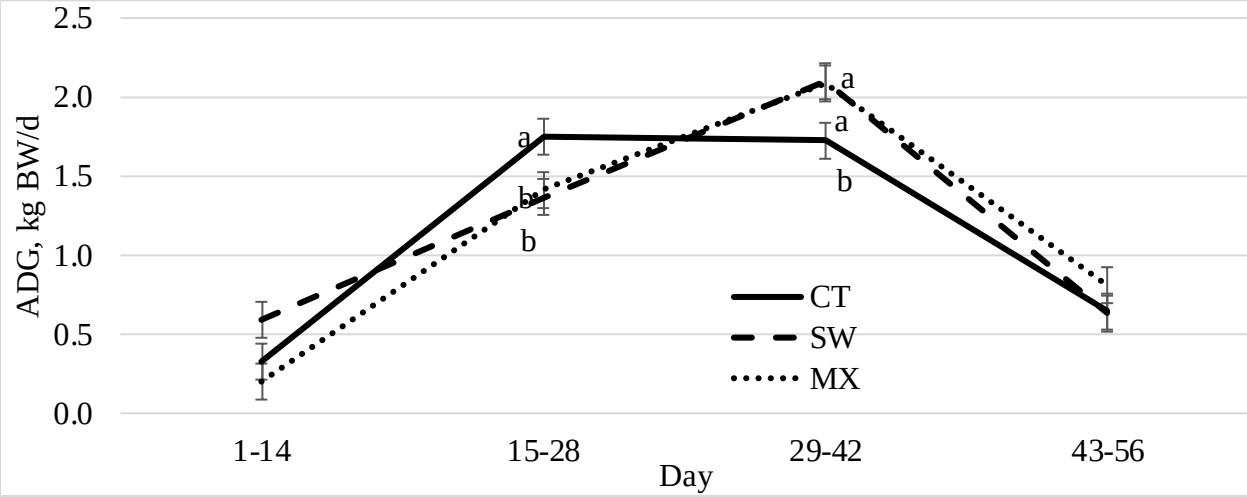


**Figure 3.1**. Average daily gain (kg BW/d) of 90 newly received steers calculated during four 14-d periods over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/t. Different letters showing significant differences ($P < 0.05$) among treatments within measuring periods.

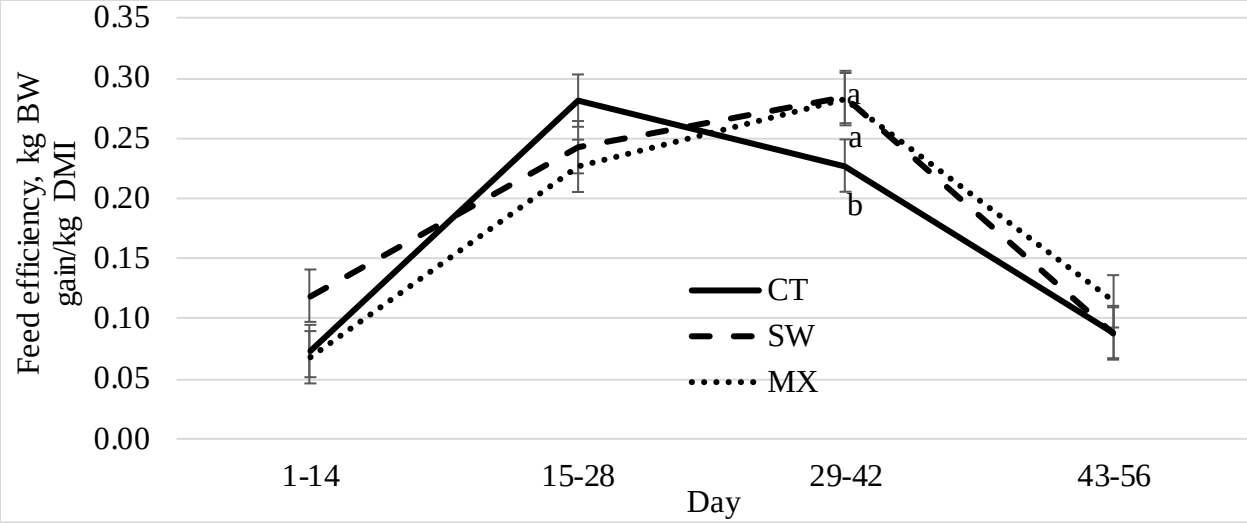


**Figure 3.2**. Feed efficiency (kg BW gain/kg DMI) of 90 newly received steers calculated during four 14-d periods over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/t. Different letters showing significant differences ($P < 0.05$) among treatments within measuring periods.

**Table 3.3**. Effects of flavouring additives on the feeding pattern of newly received steers for 56 days on three treatment groups (n=90) measured with GrowSafe (Airdrie, AB, Canada).

| | Treatments[1] | | | | P value | | |
|---|---|---|---|---|---|---|---|
| | CT | SW | MX | SEM[2] | Treatment | Week | Treat × week |
| DMI, kg/d | 6.6 | 6.7 | 6.7 | 0.24 | 0.86 | <0.01 | <0.01 |
| Daily feeding duration, min/d | 110.2 | 108.8 | 111.9 | 6.14 | 0.92 | <0.01 | <0.01 |
| Daily head down time, min/d | 42.4 | 39.7 | 43.1 | 1.08 | 0.62 | <0.01 | 0.14 |
| Meal characteristics | | | | | | | |
| Meal frequency, no./d | 16.8 | 16.5 | 17.0 | 0.64 | 0.75 | <0.01 | <0.01 |
| Meal size, g DM | 401.6 | 422.2 | 409.4 | 1.05 | 0.59 | <0.01 | <0.01 |
| Visits/meal, no. | 4.1 | 4.3 | 4.1 | 1.08 | 0.79 | <0.01 | <0.01 |
| Meal duration, min | 6.7 | 6.8 | 6.8 | 0.34 | 0.91 | <0.01 | <0.01 |
| Head down time,min/meal | 2.6 | 2.5 | 2.7 | 1.07 | 0.78 | <0.01 | 0.01 |
| Visits to the feed bunk | | | | | | | |
| Visits, no/d. | 70.2 | 72.6 | 69.4 | 4.77 | 0.85 | <0.01 | <0.01 |
| DMI, g DM/visit | 103.2 | 105.8 | 108.1 | 1.18 | 0.98 | <0.01 | <0.01 |
| Visit duration, min | 1.7 | 1.8 | 1.9 | 1.15 | 0.92 | <0.01 | <0.01 |
| Head down time, min/visit | 0.7 | 0.7 | 0.7 | 1.25 | 0.95 | <0.01 | <0.01 |
| Eating rate, g DM/min | 64.7 | 67.4 | 64.9 | 1.08 | 0.91 | <0.01 | <0.01 |
| Eating rate head down, g DM/min | 170.1 | 187.2 | 171.5 | 16.55 | 0.68 | <0.01 | 0.01 |

[1] Data are expressed as least squared means. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/Tn (both provided by Lucta SA, Barcelona, Spain).

[2] Standard error of the mean.

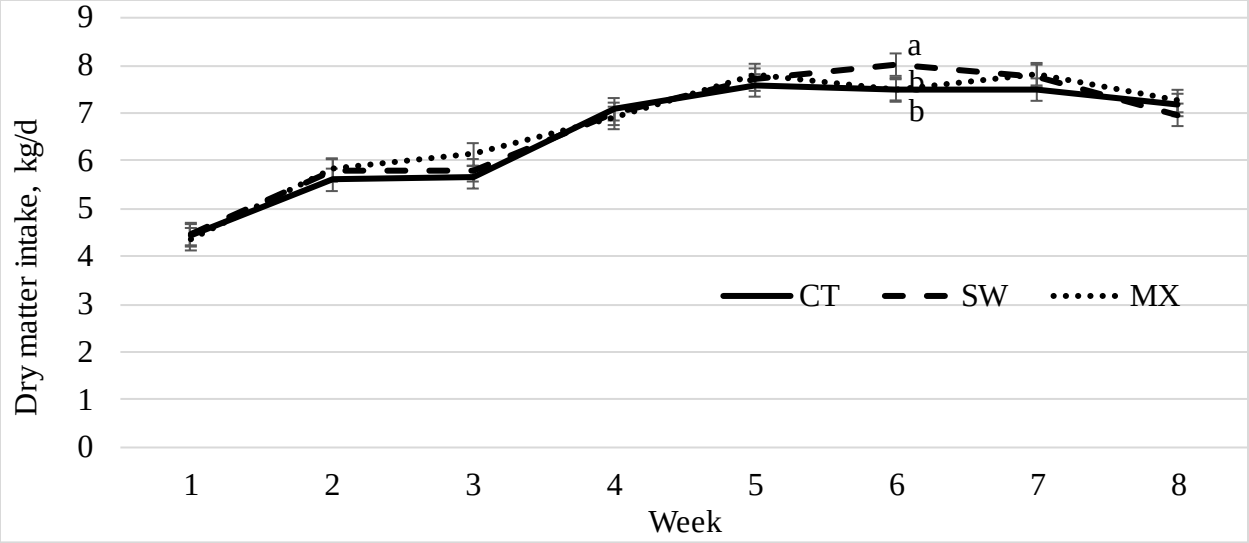


**Figure 3.3.** Weekly average of daily DMI (in kg) of 90 newly received steers over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/t. Different letters showing significant differences ($P < 0.05$) among treatments within measuring periods.

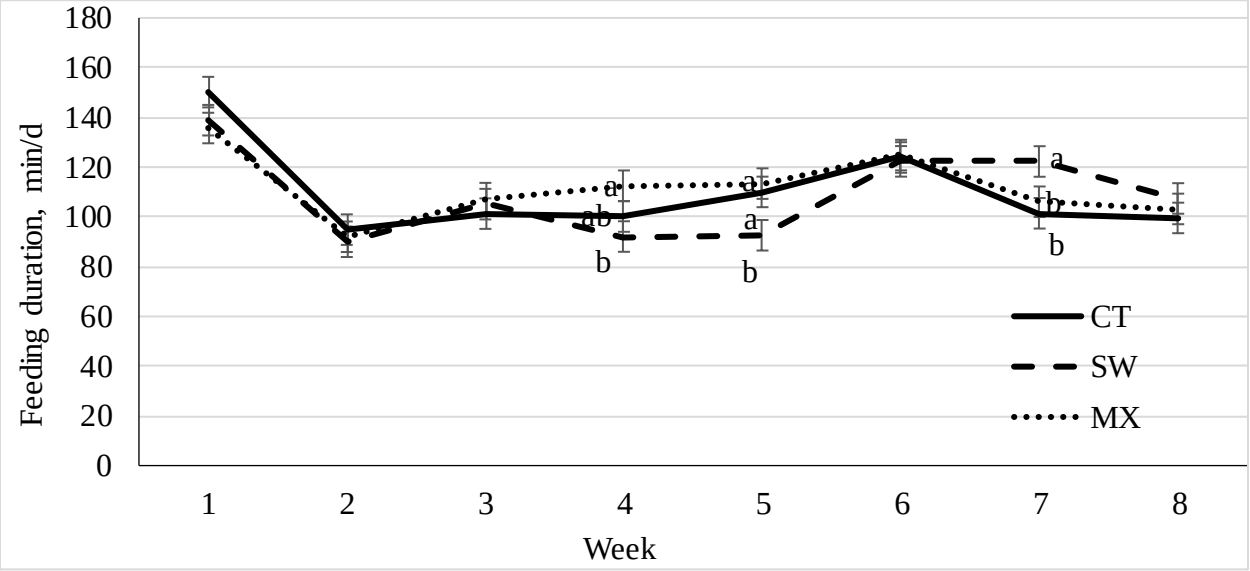


**Figure 3.4.** Weekly average of daily feeding duration (in min) of 90 newly received steers over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents

comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/t. Different letters showing significant differences ($P < 0.05$) among treatments within measuring periods.

### 3.3.2.1. Meal profile

The number of meals per day was affected by a treatment × week interaction ($P < 0.01$, Table 3.3), where it was greater in SW and MX steers than in CT steers on week 6, and it was greater in SW than in CT steers on week 7 (Figure 3.5). The meal frequency for all three diets was in the range of 14 to 19 per day.

The meal size also showed a treatment × week interaction ($P < 0.01$, Table 3.3), where it was greater in SW than CT on week 3 and greater than MX on week 4 and 5, respectively; it was greater in MX than in CT and SW steers on weeks 3 and 7, respectively (Figure 3.6).

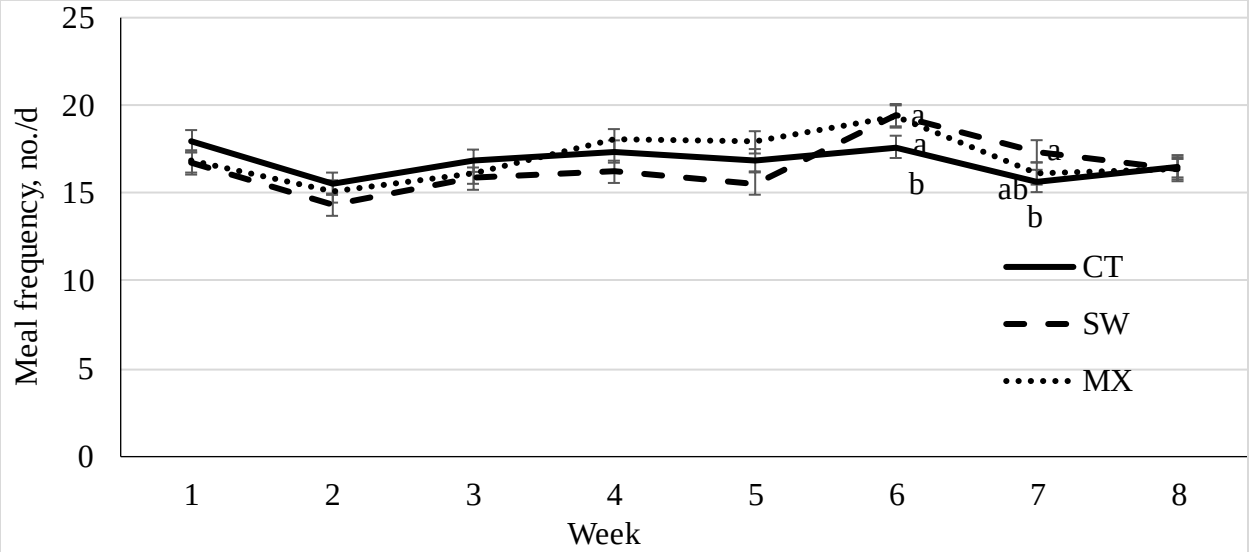


**Figure 3.5.** Weekly average of daily number of meals of 90 newly received steers over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/t. Different letters showing significant differences ($P < 0.05$) among treatments within measuring periods.

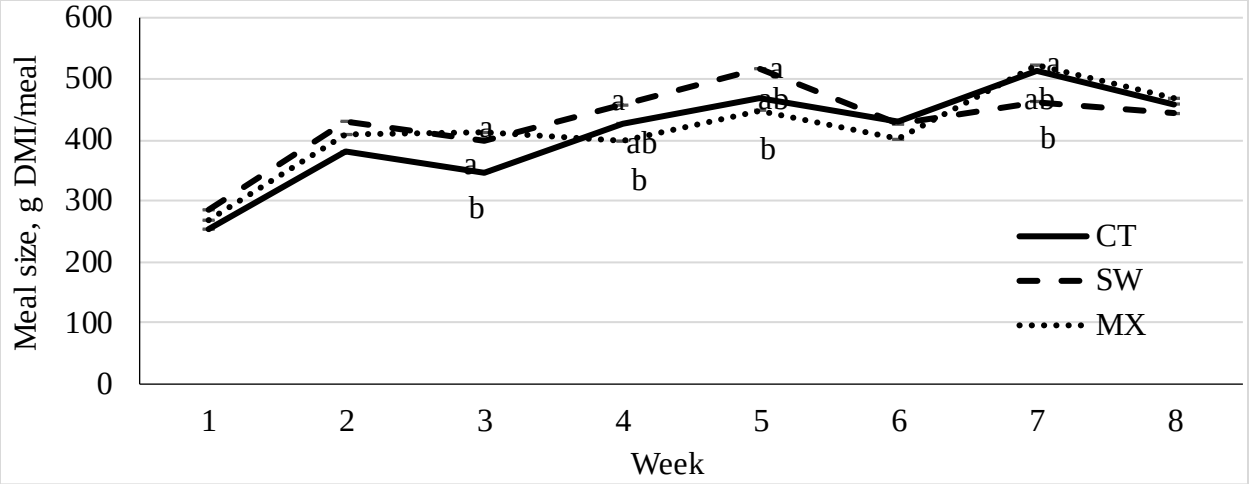


**Figure 3.6.** Weekly average of DMI per meal (meal size) of 90 newly received steers over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/t. Different letters showing significant differences ($P < 0.05$) among treatments within measuring periods.

The number of visits to the feed bunk within each meal had a treatment × week interaction ($P < 0.01$, Table 3.3), where it was greater in SW and MX than in CT steers on weeks 1 to 3; it was greater in SW than MX and, than MX and CT steers on week 4 and 5, respectively; it was greater in CT than SW steers on week 7 and 8 (Figure 3.7).

The meal duration also showed treatment × week interaction ($P < 0.01$, Table 3.3), where it was lengthier for SW steers on week 3 compared to week 2 and on weeks 7 and 8 than week 6; but it was lower on weeks 4 to 6 than week 3; and MX steers took longer time on week 3 and 4 than week 2 (Figure 3.8). The time spent with the head down while at the feed bunk within each meal showed a significant treatment × week interaction ($P < 0.01$, Table 3.3), where it was greater for SW steers on week 1 and lower on week 6 compared to the rest of the weeks (Figure 3.9).

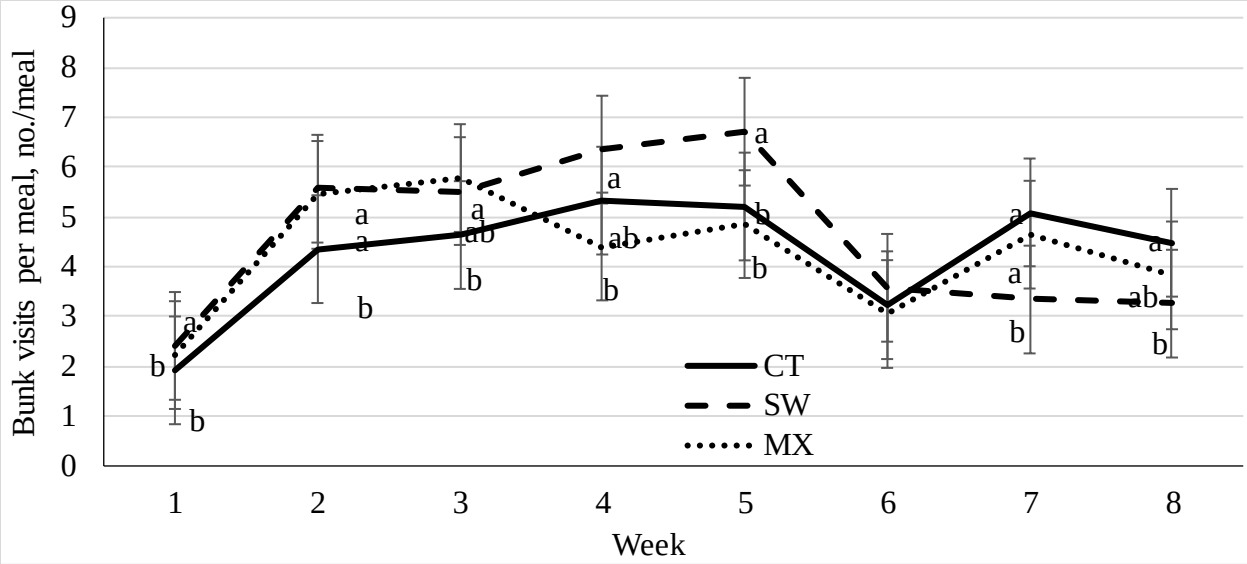


**Figure 3.7**. Weekly average of number of visits per meal of 90 newly received steers over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/t. Different letters showing significant differences ($P < 0.05$) among treatments within measuring periods.

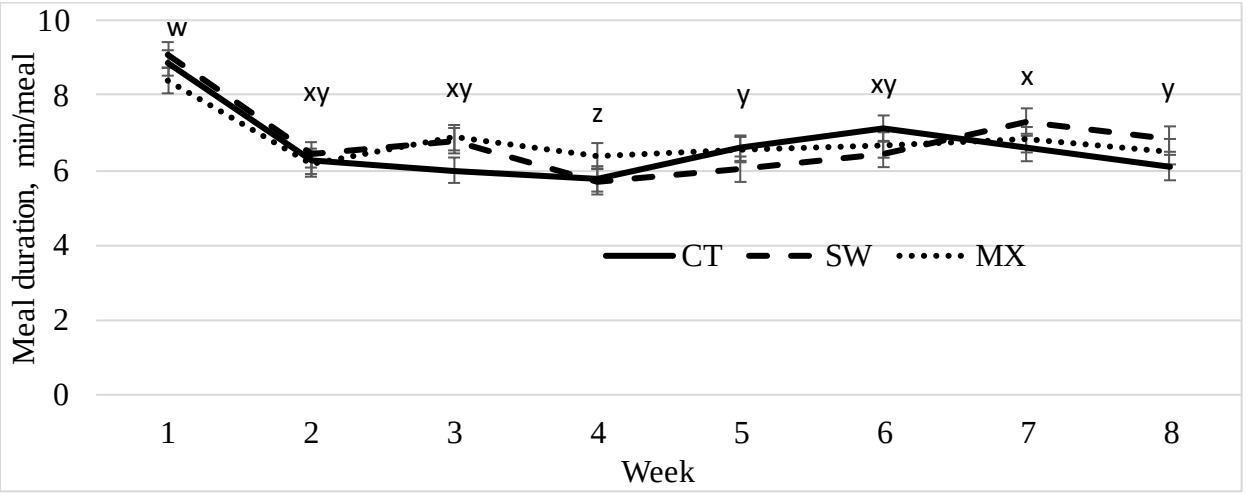


**Figure 3.8.** Weekly average of meal duration (in minutes) of 90 newly received over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/t. Different letters showing significant differences ($P < 0.05$) between weeks.

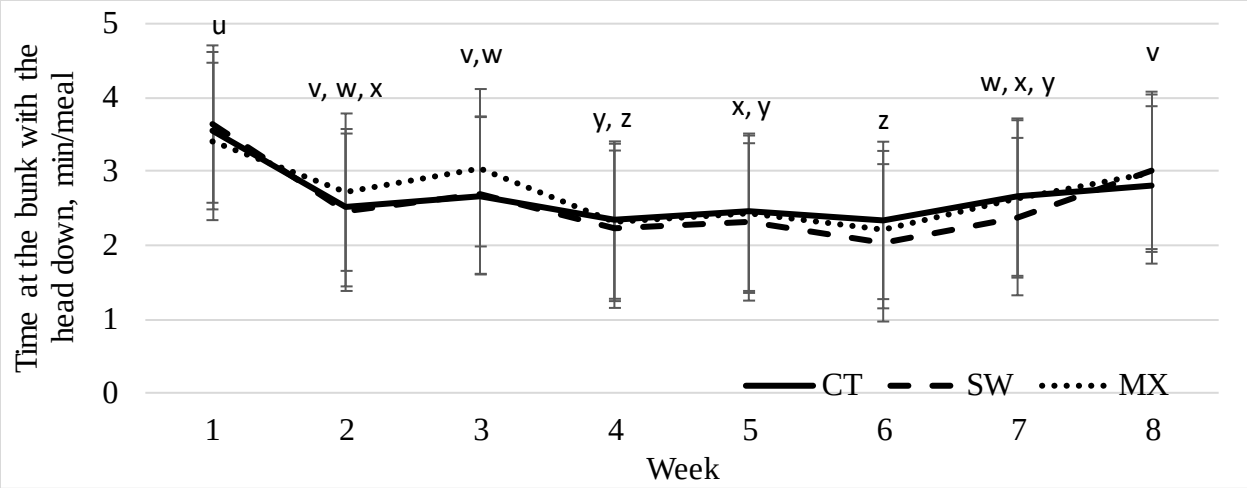


**Figure 3.9.** Weekly average of the time with the head down in the feed bunk per meal (in minutes) of 90 newly received steers over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/t. Different letters showing significant differences ($P < 0.05$) between weeks.

### 3.3.2.2. Visits to the feed bunk

The number of daily visits to the feed bunk showed a treatment × week interaction ($P < 0.01$, Table 3.3), where it was greater on MX than CT steers on week 2; it was greater in SW compared to CT and MX steers on week 4; and it was greater in CT than SW steers on week 7 and 8 (Figure 3.10).

The DMI during each visit to the feed bunk had a significant treatment × week ($P < 0.01$, Table 3.3), where it was greater on weeks 6 to 8 compared to weeks 5 in SW steers (Figure 3.11).

The duration of each visit to the feed bunk had a treatment × week interaction ($P < 0.01$, Table 3.3), where MX steers made longer visits than SW steers on week 4, while SW steers took longer time for each visit on week 7 and 8 (Figure 3.12).

The head down time of each visit also showed a significant interaction of treatment × week ($P < 0.01$, Table 3.3), where it was lower for SW steers on week 4 and 5 compared to the rest of the weeks (Figure 3.13).

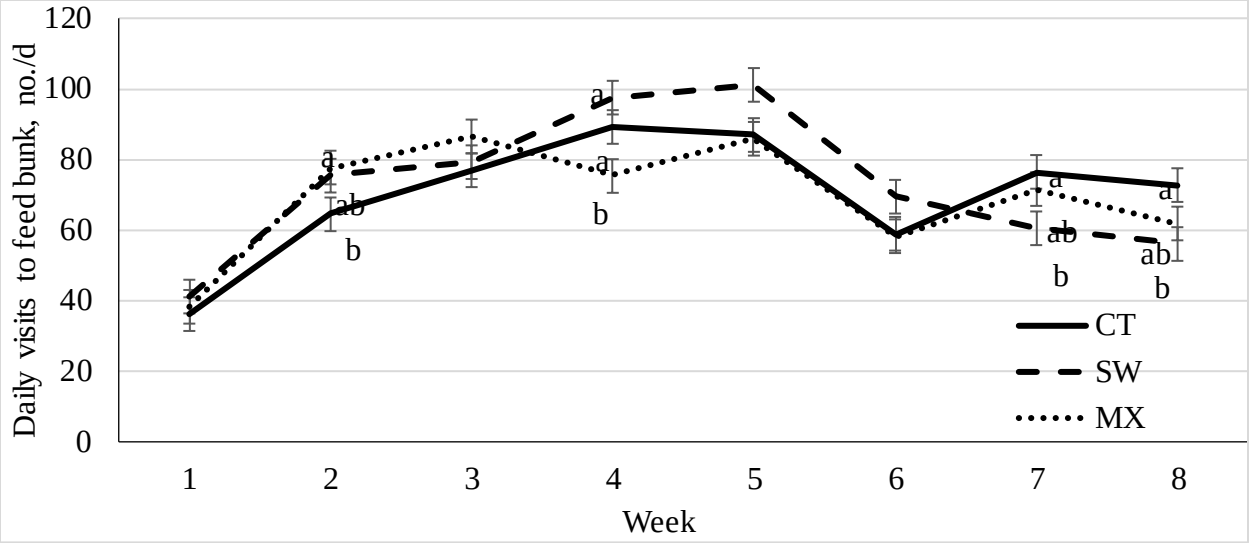


**Figure 3.10**. Weekly average of the daily frequency of visits to the feed bunk of 90 newly received steers over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/t. Different letters showing significant differences ($P < 0.05$) among treatments within measuring periods.

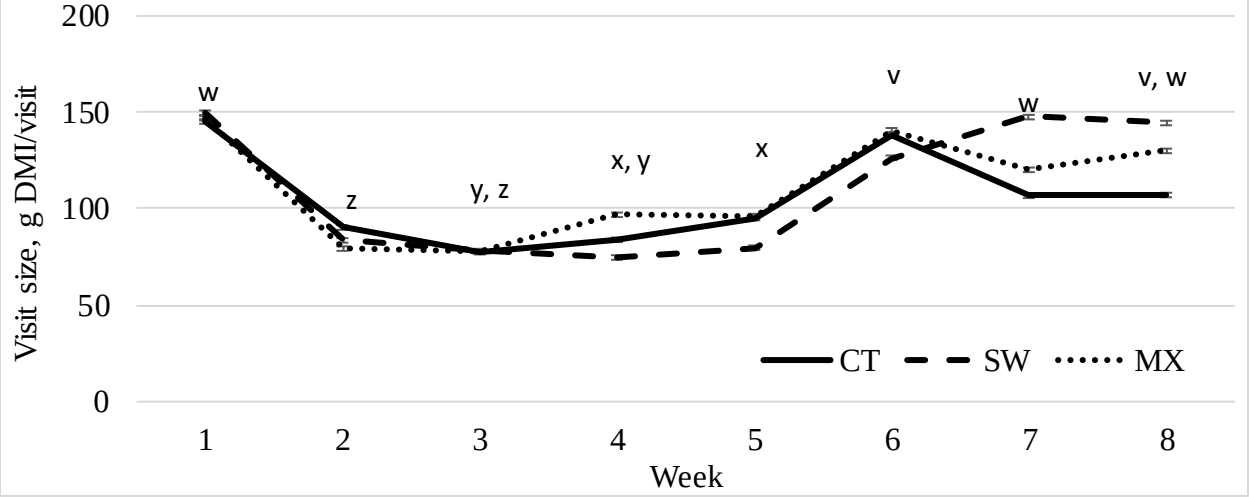


**Figure 3.11.** Weekly average of the DMI per visit of 90 newly received steers over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/t. Different letters showing significant differences ($P < 0.05$) between weeks.

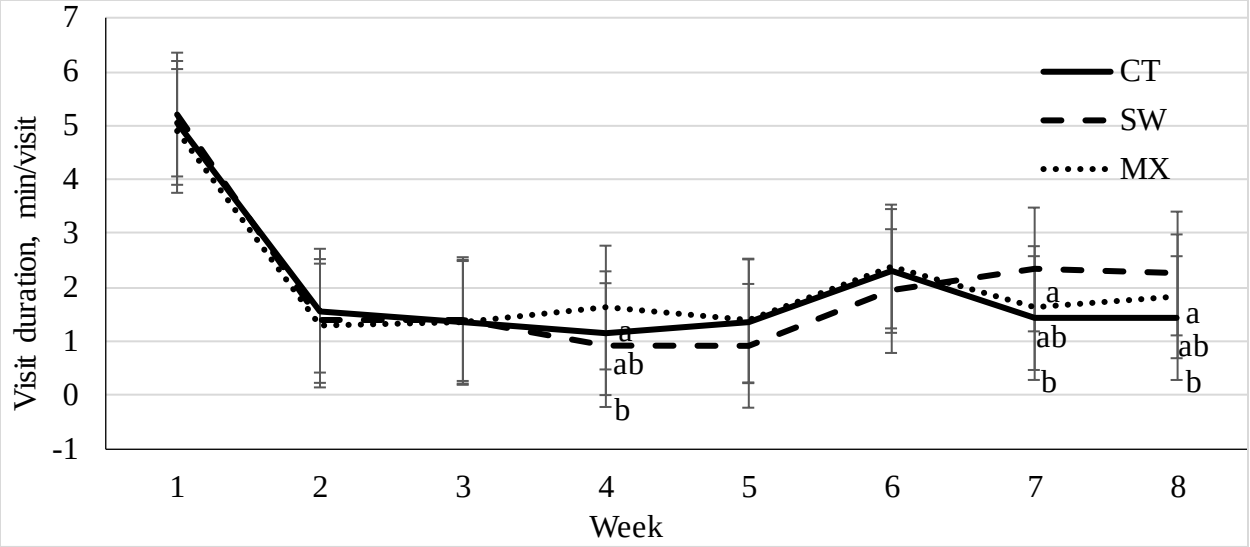


**Figure 3.12.** Weekly average of the duration of each visit to the feed bunk of 90 newly received steers over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/t. Different letters showing significant differences ($P < 0.05$) among treatments within measuring periods.

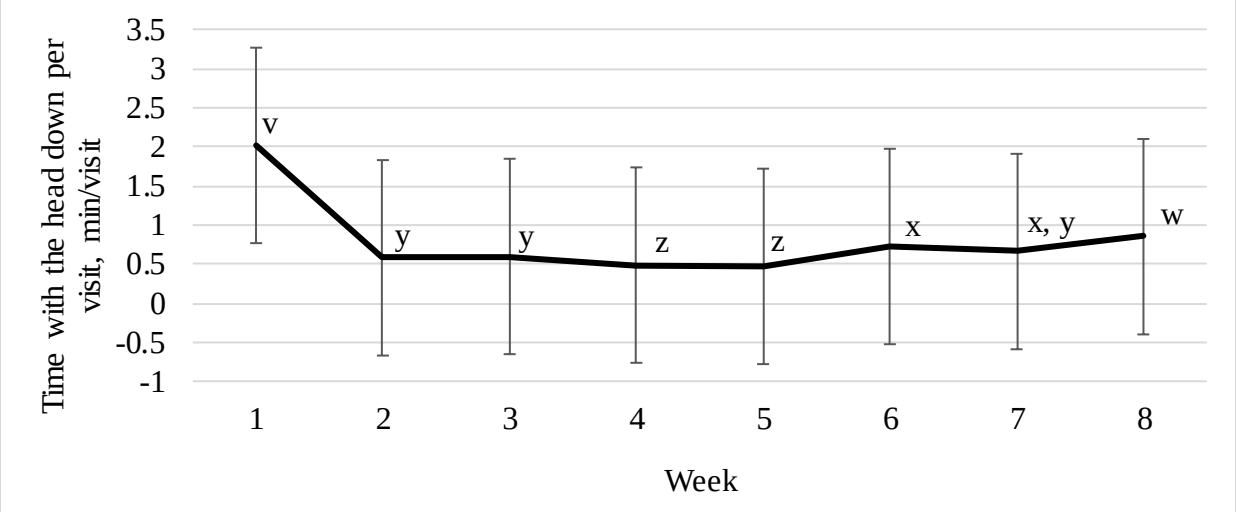


**Figure 3.13**. Weekly average of the time with the head down per visit (in minutes) of 90 newly received steers over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/t. Different letters showing significant differences ($P < 0.05$) between weeks.

### 3.3.2.3. Eating rate

The eating rate showed a treatment × week interaction ($P < 0.01$, Table 3.3), where it was greater in SW steers on week 4 and 5 compared with MX, MX and CT steers, respectively (Figure 3.14). We also noticed a treatment × week interaction ($P = 0.01$, Table 3.3) for head down eating rate, where it was lower on week 8 compared to weeks 4 to 7 only in SW steers (Figure 3.15).

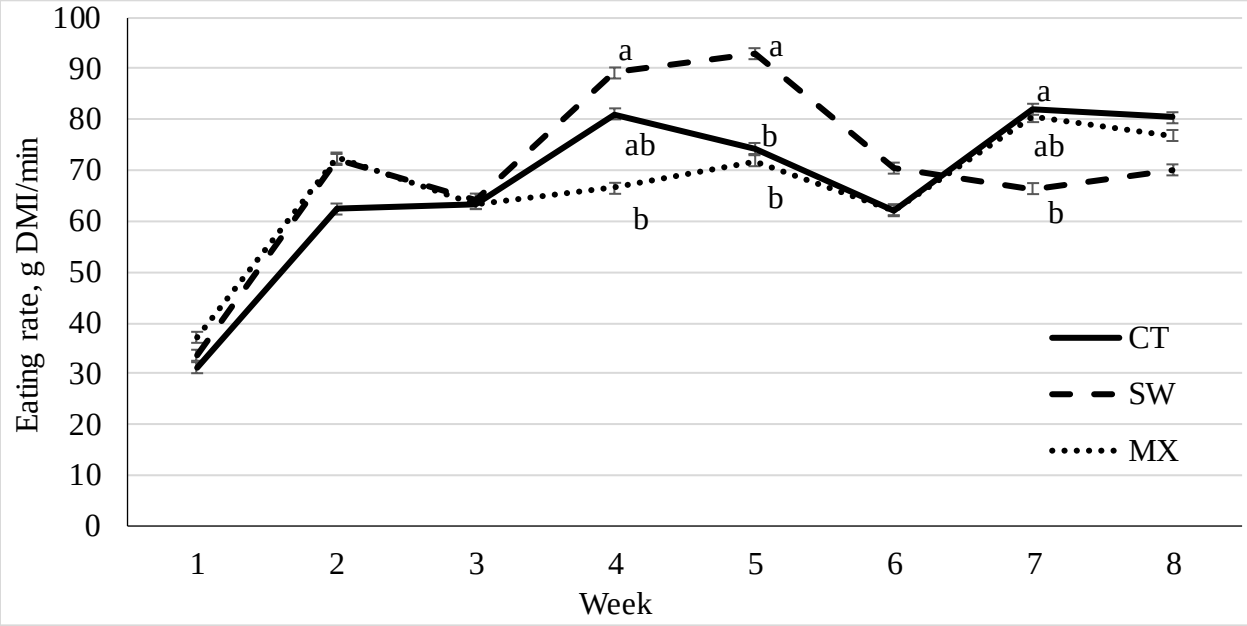


**Figure 3.14.** Weekly average of the eating rate (g DMI/min) of 90 newly received steers over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/t. Different letters showing significant differences ($P < 0.05$) among treatments within measuring periods.

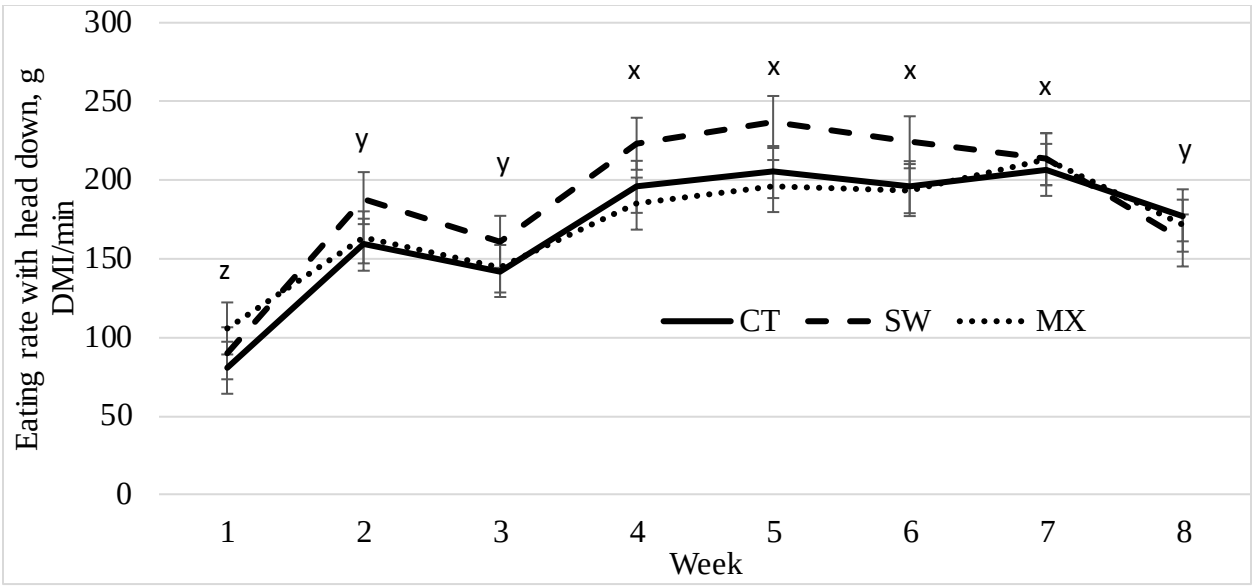


**Figure 3.15.** Weekly average of the eating rate with the head down in the feed bunk (g DMI/min) of 90 newly received steers over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/t. Different letters showing significant differences ($P < 0.05$) between weeks.

### 3.3.3. Cattle temperament

Steers showed a slower exit speed on d 28 compared with d 56 (Figure 3.16). Both the highest range of movement and the standard deviation of all the movement captured while steers where restrained in the chute tended to be lower (P = 0.06) in SW than CT steers (Table 3.4).

**Table 3.4**. Effect of flavouring additives on the chute behaviour measured with an accelerometer (in g-force, *g*) while steers (n=90) were restrained in the squeeze chute for sampling.

| | Treatments[1] | | | | *P*- value [3] | | |
|---|---|---|---|---|---|---|---|
| | CT | SW | MX | SEM[2] | CT vs SW | CT vs MX | SW vs MX |
| Total movement, *g* | | | | | | | |
| Day 1 | 149.3 | 153.07 | 147.60 | 2.36 | 0.95 | 0.40 | 0.60 |
| Day 28 | 133.9 | 126.61 | 136.35 | 4.63 | 0.84 | 0.99 | 0.97 |
| Day 56 | 134.2 | 131.43 | 134.08 | 6.61 | 0.65 | 0.95 | 0.74 |
| Average movement, *g* | | | | | | | |
| Day 1 | 0.99 | 0.99 | 0.99 | 0.00 | 0.59 | 0.22 | 0.75 |
| Day 28 | 0.99 | 0.99 | 0.98 | 0.00 | 0.43 | 0.01 | 0.28 |
| Day 56 | 0.99 | 0.98 | 0.99 | 0.00 | 0.93 | 0.94 | 0.67 |
| Highest range movement, *g* | | | | | | | |
| Day 1 | 1.35 | 1.12 | 1.14 | 0.06 | 0.57 | 0.67 | 0.98 |
| Day 28 | 1.09 | 1.11 | 1.11 | 0.03 | 0.95 | 0.96 | 0.97 |
| Day 56 | 1.43 | 1.16 | 1.31 | 0.09 | 0.06 | 0.28 | 0.70 |
| Lowest range movement, *g* | | | | | | | |
| Day 1 | 0.89 | 0.88 | 0.88 | 0.02 | 0.69 | 0.85 | 0.79 |
| Day 28 | 0.91 | 0.90 | 0.90 | 0.01 | 0.61 | 0.29 | 0.94 |
| Day 56 | 0.83 | 0.88 | 0.85 | 0.02 | 0.15 | 0.63 | 0.71 |
| Standard Deviation of movement, *g* | | | | | | | |
| Day 1 | 0.04 | 0.02 | 0.02 | 0.01 | 0.90 | 0.96 | 1.00 |
| Day 28 | 0.02 | 0.02 | 0.02 | 0.00 | 0.99 | 0.94 | 0.99 |
| Day 56 | 0.06 | 0.03 | 0.05 | 0.01 | 0.06 | 0.24 | 0.84 |

[1] Data are expressed as least squared means. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/t (both provided by Lucta SA, Barcelona, Spain).

[2] Standard error of the mean.

[3] Data normality could not be achieved, so we used non-parametric one-way ANOVA of SAS, where we applied treatment as classification variable, day for group analysis, and pairwise multiple comparison to compare the groups (30 steers per treatment).

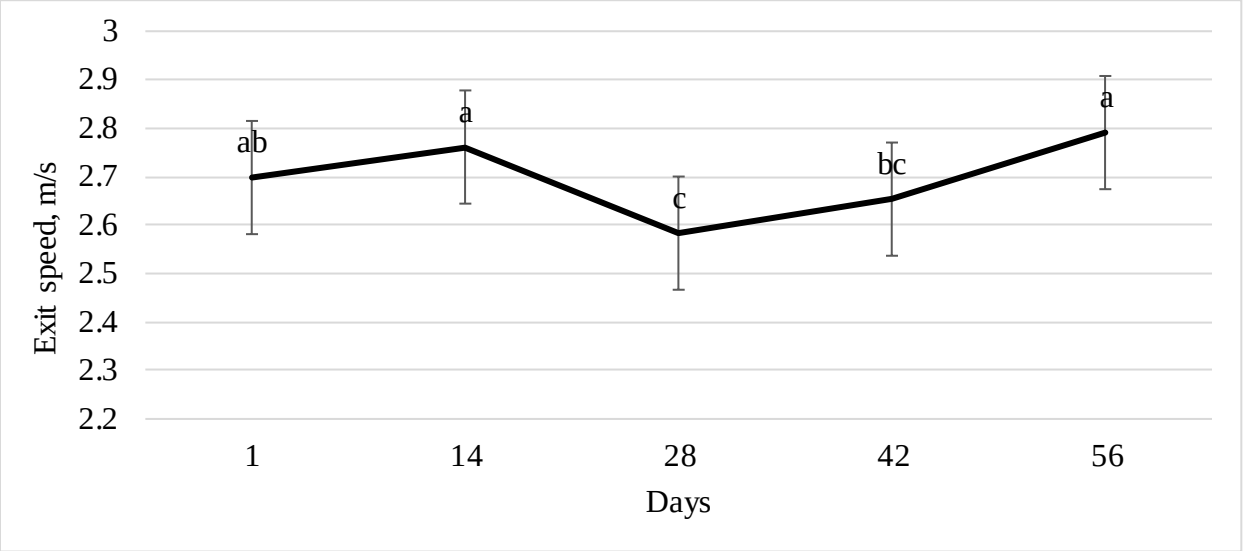


**Figure 3.16.** Average exit speed (m/s) of 90 newly received steers measured bi-weekly over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Different letters showing significant differences ($P < 0.05$) between weeks.

### 3.4. Discussion

#### 3.4.1 Growth performance, feed efficiency and DMI

The low ADG found on d 1 to 14 compared to d 15 to 42 was expected, due to the low DMI resulted from the adaptation process to the new diet and environment, as reported by Kelsey and Colpoys (2018) and Miranda et al. (2019) for the initial period of feedlot receiving heifers. The use of flavouring additives in this study did not result in a consistent effect on the growth performance of receiving steers throughout the 56-feeding period where, for example, the ADG of SW and MX steers were greater than CT on d 29 to 42, but it was lower than CT on d 15 to 28. The main effects of our treatments were hypothesized to be driven by a positive hedonic response that would stimulate DMI of receiving steers. However, the effects found on DMI were limited to SW steers having the greatest DMI on week 6. McMeniman et al. (2006) also found that a sodium saccharin-based sweetener (Sucram, Pancosma S.A., Geneva, Switzerland) increased the ADG of steers from d 28 to 56, associated with an increased DMI. Others, however, found no effect on ADG when offered sucram with either a mixture of plant extracts (cinnamaldehyde, eugenol,

capsicum; De Souza et al., 2018) or at 100, 200, or 300 g/Tn (Ponce et al., 2014) to newly received calves.

All these results suggest that the effects of flavouring additives on growth performance and DMI of cattle may vary depending on the circumstances (such as type of diet, animal, or flavoring agent) in which they are used. For example, Brown et al. (2004) reported an increased DMI in receiving feedlot steers only when sucram was added at 176 g/t compared to 0, 88 and 264 g/t, suggesting that the dose at which these flavouring additives are provided has a significant impact on the effects on DMI. Our results also suggest that the effects of flavouring additives are likely driven by other factors beyond DMI, which would include changes in the feeding pattern that may have made the digestion of the diet more efficient, or a reduced energy expenditure regarding cattle reactivity and temperament, both factors to be discussed below. The last mechanism of action that must be considered is a potential effect of the flavouring agents at a physiological level, for example, modulating rumen fermentation or because of receptor of tastes like sweet in gut which could influence on the performance of steers (Moran et al., 2014). There is good evidence (Calsamiglia et al., 2007) that some active ingredients responsible of the flavour and essence of plants (e.g. garlicin, cinnamaldehyde, or capsaicin) may have an inhibitory effect on rumen bacteria, which would change the fermentation profile of the diet ingested. Although this was not part of our objectives, and those specific active ingredients were not included in the additives used in our study, we cannot rule out the existence of this mechanism of action.

From d 29 to 42 of our study, not only the ADG but also the feed efficiency was increased by the flavouring additives, again suggesting that the treatments may not have had a major impact on DMI but rather on how the ingested diet was been used for growth. After that period, and similar to the ADG, the feed efficiency decreased for all the treatments in the last two weeks of the experiment, likely because the high values from d 15 to 42 resulted from a compensatory growth in response to the poor performance during the first 2 weeks of the study. Nevertheless, over the 56-d feeding period, there was no significant effects of the treatments on feed efficiency. McMeniman et al. (2006) found an increased feed efficiency for the first 4 weeks of newly received feedlot cattle supplemented with sucram. However, as with the ADG, Ponce et al. (2014) and De Souza et al. (2018) did not find any effect of sucram on feed efficiency of newly received feedlot steers.

### 3.4.2. Feeding behaviour

In confined or feedlot systems, feeding behaviour can be influenced by the availability of bunk space (McKinnon, 2001; González et al., 2008), cattle health (González et al., 2008), temperament (Voisinet et al., 1997), weather, and overall management strategies (Schwartzkopf-Genswein et al., 2003; Schwartzkopf-Genswein et al., 2004; Schwartzkopf-Genswein et al., 2011b). However, to the best of our knowledge, there are no studies on the effect of flavouring additives on the feeding pattern of feedlot cattle. Given limited research in this area, the discussion will include publications focusing on feeding behaviour but with different type of additives. In the current study, we used a total of 14 feeding behaviour parameters to understand how the feeding pattern may have been influenced by flavouring additives.

The time spent at the feed bunk was reduced in all treatments from week 1 to 2, likely because of the change from the adaptation diet to standard receiving diet (Schwartzkopf-Genswein et al., 2011b), which had a slightly greater energy content, but most importantly, a lower proportion of forage, which reduces the time spent for feed apprehension and chewing (Kononoff and Heinrichs, 2003). After that, from wk 5 to 7 SW steers increased their time spent at the feed bunk, while CT and MX steers remained relatively constant. Other studies evaluating different additives, such as pine enhanced biochar (Terry et al., 2019) or probiotics (Miranda el at, 2019a) have found no effects on the meal duration of cattle. Time spent at the feed bunk is positively correlated with daily dry matter consumption and average daily gain (Schwartzkopf-Genswein et al., 2002), so, it is likely that the increase in DMI seen in SW steers happened due to an associated increase in their time spent at the feed bunk. Similar to the DMI, feed efficiency has also been found to be correlated with the time spent at the feed bunk, but in this case negatively correlated (Schwartzkopf-Genswein et al., 2002). Miranda et al. (2019a), however reported that the correlation between feed efficiency and time spent at the feed bunk depends on the type of the diet. In our study, with steers feeding a standard receiving diet, the increased time spent at the feed bunk by SW steers took place over the same period of time that those steers had the greatest feed efficiency and ADG compared to CT (d 29 to 42), although these differences between treatments were not significant when considering the whole 56-d feeding period. This suggests that the effects of SW treatment could at least be partially attributed to the increased time spent at the feed bunk, although this effect was masked or compensated when considering the whole 56-d feeding period.

Daily meal frequency was also greater in SW and MX steers than in CT on week 6, and in SW steers than CT on week 7. This increase was likely connected with the same SW steers spending more time at the feed bunk and eating more. All these effects are compatible with a positive hedonic response associated with SW that would stimulate the steers to make eat more frequently. To the best of our knowledge, the effects of other additives have not been tested on meal frequency, but the use of alternative feed ingredients (corn by-products; Holtshausen et al., 2011) or feeding management strategies (da Silva et al., 2018) showed no effects on meal frequency of beef cattle. Overall, an increased number of eating bouts (whether that is a greater number of daily meals or visits to the feed bunk) is considered to be a desirable trait to optimize the rumen fermentation profile, as it warrants a more constant provision of energy to the rumen microbiota and attenuates the variability of rumen pH (González et al., 2008). This would explain the greater feed efficiency of SW and MX steers over the same period of time that had the greatest meal frequency. Similarly, Robinson and Oddy (2004) found a negative correlation between eating sessions and feed conversion ratio (kg DMI/kg BW), although also noting large differences between breeds and sex, and receiving calves not being part of the analysis. Finally, the meal frequency for our three treatments was in the range of 14 to 19 per day and it is consistent with that reported by Holtshausen et al. (2011) and da Silva et al. (2018). It is worth to note that these two studies used the method described by Yates et al. (2001) to define the meal criteria, instead of the 300-sec rule that we used, suggesting that the methodology used for such calculation did not have a major impact on the results.

The use of SW and MX increased the frequency of visits to the feed bunk per meal compared to CT on weeks 1 to 3, and on week 5 for SW steers. At a later stage of the study, however, CT steers made more visits per meal than SW steers. The daily frequency of visits to the feed bunk followed a similar pattern, where on week 2 SW and MX steers visited the feed bunk 17% and 20.6% more often than CT steers, respectively; on week 4 SW steers showed 29.5% and 9.3% higher number of visits than MX and CT steers, respectively; and on week 5, SW went 17.8% and 16.3% more times to the feed bunk than MX and CT steers, respectively. Again, at a later stage of the study, either the CT steers had a compensatory effect, or the effect of the flavouring additives fade away, but CT steers visited the feed bunk 26.4% more often on week 7 and 29.8% on week 8 than SW steers. These results suggest that the hedonic effects of the additives could have stimulated MX and SW steers to visit more often the feed bunk, mostly in the first few weeks

of the 56-feeding period, when steers may have found the flavour a novel and interesting stimulus. The hedonic component of the diet is recognized to interact with other homeostatic mechanisms involved in appetite control (Dalton and Finlayson, 2013), and Villalba et al. (2011) also showed that lambs exposed to diverse flavours has the potential modulate feeding behaviour by inducing a more even consumption of feed across time, reducing peaks and nadirs of intake, compared with exposure to unflavoured rations. In our study, however, these effects were not consistent over the 56-d feeding trial, suggesting that the positive hedonic response to the added flavours may have been override over time by the fact that cattle were eating a single food (the TMR) and a single flavour profile to satiety, which causes the phenomenon known as "sensory-specific satiety". Further research is necessary to better understand the short- and long-term effect on the feeding pattern of cattle offered one or a diverse flavouring profile.

The effects of our treatments over the weeks (mostly from wk 4 to 8 of the study) on the frequency of visits per meal were similar to those found on the eating rate, and inversely related to those on the duration and size of the visits. This means that, in general terms, till wk 6 SW steers were making the greater number of visits per meal, and those visits were the shortest and smallest, while steers were eating the fastest compared to the other treatments. After wk 6, SW steers started to visit the bunk less often and eat slower, so those visits became longer and bigger. All these changes are compatible with a greater competitive environment at the feed bunk on wk 4 and 5 for SW steers, and this competition becoming less intense towards the end of the study, while MX and CT steers seemed to have a more stable feeding behaviour over the length of the study. Again, this could be a result of the positive hedonic response caused by the SW treatment, which peaked after one month, and then the effects may have faded away as the stimulus became less effective. As mentioned above, it was hard to find a biological explanation for this effect other than the interaction of the hedonic aspects of the diet with mechanisms involved in appetite control and satiety (Dalton and Finlayson 2013). The lack of consistency during the 56-d study, however, may indicate that steers may have got used to such stimulus over time, supporting the presence of some level of sensory-specific satiety, as suggested above. More research on this area is necessary to provide a better understanding of the hedonic response to TMR diets in feedlot cattle.

Another factor to consider when interpreting our feeding behaviour data is the greater variability associated with steers adapting to the diet, feed bunks and pen mates. In our case, this

would have been exacerbated by the use of the GrowSafe bunks, which were effectively reducing the feeding spaces to 1 bunk per every 5 steers. Moreover, the animals were not provided with a period of adaptation to the bunks as we wanted to capture the effect of treatments as soon as they entered in the feedlot. As a result, for example, the eating rate that was supposed to be greater as body weight increases (Moya et al., 2011), seemed to be artificially inflated in SW steers after wk 4 and 5. A similar result was reported by Haskell et al. (2019) when they offered 1 feed bunk space for every 2.5 finishing beef steers, as they described that the competition for a bunk space started just after the feed was delivered, likely because of the attraction for fresh feed, easiness to eat from a bunk full of feed, and their natural motivation for cattle to eat as a group and within the first few hours of the day.

We found no clear connection between the frequency and length of the visits to the feed bunk and steer performance but Schwartzkopf-Genswein et al. (2011) found that the frequency of visits was lower and the length was greater in highly efficient animals with greater ADG compared to average or low efficient animals during the backgrounding and finishing period.

We also found no major effects of the treatments on meal duration and frequency over the study except a higher meal frequency on wk 6 and 7 for SW and SW and MX, respectively; but the meal size was 15% and 19% greater in SW and MX than CT on wk 3, respectively; 14.7% and 15% greater in SW than MX on week 4 and 5, respectively; and 13% greater in MX than SW on week 7. These changes in the meal size are likely the consequence of a combined effect of the increased frequency of visits and eating rate, which resulted in larger meals, although the differences were not big enough as to have a significant impact on total DMI. Again, this suggests that the flavouring additives had an impact on the way feed was consumed by the steers, likely because of its hedonic response on the steers. Others have found no effects on meal size with different feed delivery times (da Silva et al., 2018) or different energy sources for barley grain replacement (Holtshausen et al., 2011).

### 3.4.3. Temperament

Palatable feeds have shown a promising effect on reducing the behavioural and physiological responses to stress on rats (Ulrich-Lai et al., 2007). At a neurophysiological level, highly palatable substrates have a similar effect than psychostimulants on releasing dopamine (Pelchat, 2009), which may serve to shape the response to stress stimuli. Ultimately, it was

hypothesized that the addition of flavours could increase feed intake by the effect of hedonic value of taste (influencing on animal to visit frequently and consume more), which it would reduce the negative impact of steers transitioning to the feedlot environment contributing to having a calmer herd. The results from our study showed no effects of the flavouring treatments on the exit speed, but SW tended to reduce both the maximum force exerted and variability of the movement captured while steers were restrained in the chute, suggesting that SW steers had a calmer reaction to that scenario. This calmer temperament could be linked to the greater growth performance and efficiency described above, as previously described by Bruno et al. (2016). The lower exit speed on d 28 compared to d 56 does not have a clear biological explanation, but previous studies have attributed the variation in exit speed over time to a nonlinear display of the trait over time (Müller and von Keyserlingk, 2006). Moreover, weather events (e.g. snow) or changes in the personnel handling the steers may have contributed to these differences between weeks.

### 3.5. Conclusions

The flavouring additives showed some effects on the behaviour of newly arrived feedlot cattle, as seen by a changing pattern on frequency, size and length of the visits to the feed bunk, as well as on the eating rate and chute behaviour at specific weeks of the experiment. These effects resulted in differences in the feed efficiency and ADG, although these were limited to specific periods of the experiment. Overall, the use of flavouring agents did not cause a clear benefit on the performance and welfare of receiving steers. Future research should address whether the episodic changes in feeding pattern, performance and temperament are biologically and economically relevant by improving the resilience of calves to the feedlot receiving period.

# 4. Effect of flavouring additives on the immune function and biomarkers of inflammation of newly received feedlot cattle

## 4.1. Introduction

The stress and feed deprivation experienced by calves during weaning (Boland et al., 2008) and transportation (González et al., 2012) has been associated with a compromised absorptive and barrier function of the gastrointestinal tract (GIT), linked to an increased inflammatory response and impaired the immune function (Goff, 2006; Albornoz et al., 2013; Zhang et al., 2013). Moreover, a greater incidence of bovine respiratory disease upon entry to the feedlot has been linked to the stress levels of calves, along with the exchange of pathogens while commingling in trailers and/or auction markets (Hodgson et al., 2012).

Different physiological parameters have been studied to assess the welfare and health status of receiving cattle. Increased concentration of blood cortisol prior to and following transportation has been associated with human-animal interactions and microclimate conditions in the trailer (Burdick et al., 2011a) and to feed restriction before and during the transportation (Bourguet et al., 2011), which confirms that these procedures cause stress to the calves. Also, increased concentrations of acute-phase proteins (APP) have been determined in response to pulmonary and/or GIT tissue damage, inflammation and stress (Ceciliani et al., 2012; Tothova et al., 2014).

Flavouring additives can modulate the hedonic response in calves, thus potentially stimulating their feed intake (Baumont, 1996; Montoro et al., 2011; Yang et al., 2010), and hence preventing or reducing the negative consequences of the transition to a feedlot environment. We hypothesized that the use of flavouring additives will cause changes in the feeding pattern of newly received feedlot steers that will promote an improved immune function, lower cortisol levels, and a reduced inflammation response.

## 4.2. Materials and methods

This work was approved by the University of Saskatchewan's Animal Research Ethics Board and adhered to the Canadian Council on Animal Care guidelines for humane animal use (animal use protocol: 20190123). The experimental phase of this study was conducted from November 2019 to January 2020 at the Livestock Forage and Excellence Centre (LFCE) of the University of Saskatchewan (Saskatoon, Canada).

### 4.2.1 Animals used

The samples to complete this chapter were collected on the same steers as described in Chapter 3. Briefly, 90 Angus × Hereford steers (253.7 ± 36.7 kg BW) were homogenously assigned based on BW to 6 pens (15 steers per pen). Three treatments were then assigned to those pens (2 pens per treatment, ensuring that two adjacent pens did not have the same treatment): a standard feedlot receiving diet (control, **CT**; Table 1); or the same diet with flavouring additives comprised of either sweeteners (Luctarom Feedlot, **SW**) or a mix of basic tastes (Luctarom Feedlot Mix, **MX**) at 1 kg/t (both provided by Lucta SA, Barcelona, Spain). Diet composition was designed to meet or exceed NRC recommendations.

### 4.2.2 Sampling procedures

Blood samples were collected from jugular vein (Vacutainer) randomly from 7 steers per pen at day 1, 14, 28, 42 and 56 of the experiment. In total, 3 tubes were used in each animal: one 10-mL tube without a blood clotting agent and two 4-mL tubes with anticoagulant ($K_2$-EDTA). Blood samples were stored on ice and transferred immediately to the University of Saskatchewan for the rest of the procedure.

The clotted blood samples were centrifuged at 1,435 *g* for 20 minutes at 4°C. The supernatant (serum) was then transferred to free standing 1-mL microcentrifuge tubes in 0.5-mL aliquots. The serum samples were then stored at -80°C until processed for analysis. One of the 4-mL tubes with anticoagulant was used for plasma separation following the same protocol as described for the serum collection.

#### 4.2.2.1 Immunoglobulin M

Serum IgM was analyzed with a commercial ELISA kit (Abnova bovine IgM EIISA kit, Neihu District, Taipei City, Taiwan) following the manufacturer's specifications. Briefly, all the supplied reagents were diluted to be used within kit's detection range. After placing serum samples and standards (provided with the kits), the ELISA plates were incubated in darkness at room temperature and washed, and the enzyme-antibody conjugate was added followed by the addition of substrate solution and stop solution. The absorbance of the content of each well was read by a 450 nm Microtitre plate reader (BIO-RAD Laboratories Inc., Hercules, California, USA). Finally, a 4-parameter logistic curve fit was used to calculate the concentration of IgM in µg/mL.

#### 4.2.2.2. Interleukin 6

Serum interleukin 6 was also analyzed with a commercial bovine ELISA kit (R&D Systems, Inc. Minneapolis, Minnesota, USA). The ELISA plates were supplied uncoated, so we needed to coat the 96 well plates overnight following the manufacturer's indications before running the kits. Similar as with the IgM protocol described above, plates were read with a 450 nm Microtitre plate reader (BIO-RAD Laboratories Inc., Hercules, California, USA), and a 4-parameter logistic curve fit was applied to detect the concentration level of IL-6 as pg/mL.

#### 4.2.2.3. Haptoglobin

Serum haptoglobin was quantified with a commercial ELISA kit (Bovine haptoglobin ELISA kit, NOVUS Biologicals, Littleton, Colorado, USA) following the procedure described in the manual provided with the kit. Briefly, after 50 μL of standards and samples were added in the coated wells and incubated for 2 hours. Then, haptoglobin antibody was added followed by conjugate, chromogen substrate, and stop solution. After each addition the plates were incubated in darkness at room temperature and washed. Later, we read the absorbance on a microplate reader (BIO-RAD Laboratories Inc., Hercules, California, USA) at a wavelength of 450 nm, and the concentration of haptoglobin was extracted in mg/ml using a 4-parameter logistic curve fit.

#### 4.2.2.4. Serum Amyloid A

Serum amyloid A (SAA) was measured using a commercial bovine ELISA kit (Life Diagnostic, Inc. West Chester, PA, USA). Serum samples were first denatured at 60º C in a water bath for an hour and then followed the protocol described in the manual. Plates absorbance were read with 450 nm Microtitre plate reader (BIO-RAD Laboratories Inc., Hercules, California, USA), and the concentration of SAA was extracted in ng/mL using a 4-parameter curve fit.

#### 4.2.2.5. Fibrinogen

Plasma fibrinogen was determined with a commercial ELISA kit (Bovine Fibrinogen Elisa kit, Cusabio Technology LLC, Wuhan, Hubei, China). The procedure was followed as described on the manufacturer's specifications. Briefly, plasma samples were placed along with standards on the coated ELISA plates. Then, the conjugate was added and incubated for 40 min at 37ºC. Later, the procedure includes washing the plates 4 times, adding 90 μL of TMB substrate, and finishing with 50 μL stop solution. We then read the plates under a plate reader (BIO-RAD

Laboratories Inc., Hercules, California, USA) at 450 nm to get the raw data. The concentration of fibrinogen was then calculated in mg/mL applying a 4-parameter logistic curve fit.

#### 4.2.2.6. Blood smear

The remaining 4-mL tube with anticoagulant was used for making a blood smear. With the help of automatic pipette, 600 μL of blood were poured on a glass slide. Then with the edge of a second glass slide held at a 45°, the drop of blood was pushed in such a way that the blood covered the maximum space of the first glass slide. We then kept the smear on a tissue paper for air drying. After air drying, the slide was dipped repeatedly into a fixative for a minimum of 5 seconds, after which the excess fixative was drained off. The same procedure was repeated for the stain solution, and the counter stain. The stains used were a mixture of eosin and methylene blue. Finally, the slides were washed with distilled water and dried on a paper towel. Slides were read under the microscope at 100 augments to count in a total of 100 cells from each slide, differentiating 5 types of WBC (neutrophils, lymphocytes, monocytes, basophils, and eosinophils).

#### 4.2.2.7. Salivary cortisol

Saliva samples were collected on day 1, 14, 28, 42 and 56 of the experiment by rubbing a cotton swab on the floor of the buccal cavity of the steers. The cotton swabs used had been wrapped with additional cotton to increase the amount of liquid to be captured. Samples were then kept inside 15-mL Falcon tubes and stored immediately at -20°C until analysis. Before doing the ELISA, tubes were centrifugated at $918 \times g$ for 15 minutes to separate the saliva from the cotton swab. Analysis of the saliva samples was done with a commercial ELISA kit (Salimetrics ELISA, PA, USA), following the manufacturer's specification. A plate reader was used to detect the absorbance value of each well at 450 nm with a second correction of 490 nm. Using a 4-parameters logistic curve, the concentration of salivary cortisol was obtained.

#### 4.2.2.8. Hair cortisol

Hair samples were collected on d 1, 28 and 56 of the study from the hip area of steers with the help of an electric clipping machine as described by Moya et al. (2013). The area clipped was approximately $10 \times 10$ cm, for a targeted volume of hair sample of 200 mg, which were stored in paper envelopes at room temperature until they were analyzed. Cortisol extraction of hair was performed as described by Moya et al. (2013). Briefly, hair samples were cleaned with methanol of 3 times and left to dry for a minimum of 24 hours. Using a mixer mill (Retsh mixer mill MM

400), 100 mg of dried hair samples were ground to fine particles during a predetermined time based on the volume of hair of each sample.

Methanol was then added to 25 mg of grounded hair samples and incubated in a rotatory incubator for 24 hours. The supernatant was then extracted and dried under a gentle stream of nitrogen gas. Later, samples were reconstituted with the assay diluent and transferred to a fresh snap cap Eppendorf tube, where it was kept at -20ºC until analyzed using a commercial ELISA kit (Salimetrics Elisa, State College, PA, USA) following the manufacturer's specification. A plate reader was used to detect the absorbance value of each well at 450 nm with a second correction of 490 nm. Using a 4-parameters logistic curve fit, the concentration of salivary cortisol was obtained.

### 4.2.3. Statistical analysis

All statistical analyses were completed using SAS OnDemand for academics® (SAS institute Inc., SAS Campus Drive, Cary, North Carolina, USA). All parameters were checked for normal distribution using the Proc Univariate of SAS. Normality of the data was assessed based on the histogram for data distribution, tests for normality, the curve of normal quantiles, and plot of residuals. Most parameters were found to be not normally distributed, except for the lymphocyte percentage and haptoglobin levels, so a log transformation ($log_{10}$) was applied to achieve normality. Inverse square root had to be applied for saliva and hair cortisol concentration. Inflammatory mediators, WBC differential, and cortisol concentration parameters were analyzed using a mixed model for repeated measures, including treatment, day, and its interaction as fixed effects, day as the repeated measure, pen as random effect and steers were considered as the subject. An unstructured covariance parameter was applied for plasma fibrinogen; a compound symmetry for serum amyloid A, IL-6, and neutrophil percentages; a heterogenous compound symmetry for serum IgM and lymphocyte percentages; and an ante-dependence for haptoglobin and salivary cortisol concentration after comparing Akaike Information Criterion and Bayesian Information Criterion as the best fit statistics for the tested variables. The Tukey method was used as a multiple comparison test and the significance level was declared as $\underline{P} < 0.05$. All data presented in tables and figures are LS means and SEM obtained from the SAS output, except for those variables which raw data had to be transformed to achieve normal distribution, in which case the LS means were reverse transformed for a better interpretation of the presented results. No

standard error of means has been showed for saliva and hair cortisol as the value is larger than the cortisol concentration level.

### 4.3. Results

The concentration of IL-6 was greater ($P < 0.01$) on d 1 and 28 than on d 14, 42, and 56 (Table 4.1; Figure 4.1). The IgM concentration was greater ($P < 0.01$) on d 1 compared to other measuring periods (Figure 4.2).

**Table 4.1.** Effects of flavouring additives on the biomarkers of inflammation, acute phase proteins, and white blood cells differential of newly received feedlot steers (n=42) during the 56-days feeding period.

| | Treatments[1] | | | | P-value | | |
|---|---|---|---|---|---|---|---|
| | CT | SW | MX | SEM[2] | Treatment | Day | Treat × Day |
| Biomarkers of inflammation | | | | | | | |
| IL-6, pg/mL | 83.7 | 77.6 | 73.9 | 0.14 | 0.91 | <0.01 | 0.75 |
| IgM, µg/mL | 1733.8 | 1516.0 | 1759.5 | 0.04 | 0.17 | <0.01 | 0.51 |
| Immune response (acute phase proteins) | | | | | | | |
| Haptoglobin, ng/mL | 0.8 | 0.7 | 0.8 | 1.76 | 0.05 | <0.01 | <0.01 |
| SAA[3], ng/mL | 458.9 | 365.3 | 595.1 | 0.10 | 0.10 | <0.01 | 0.31 |
| Fibrinogen, mg/mL | 2.5 | 2.5 | 2.4 | 0.04 | 0.93 | <0.01 | 0.74 |
| White blood cells differential | | | | | | | |
| Lymphocytes, % | 69.0 | 67.7 | 69.0 | 1.26 | 0.26 | <0.01 | 0.33 |
| Neutrophil, % | 25.7 | 27.4 | 25.9 | 0.02 | 0.11 | <0.01 | 0.56 |
| L:N ratio | 2.7 | 2.5 | 2.7 | 0.03 | 0.13 | <0.01 | 0.45 |

[1] Data expressed as least square means. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/Tn (both provided by Lucta SA, Barcelona, Spain).

[2] Standard error of the mean.

[3] Serum Amyloid A.

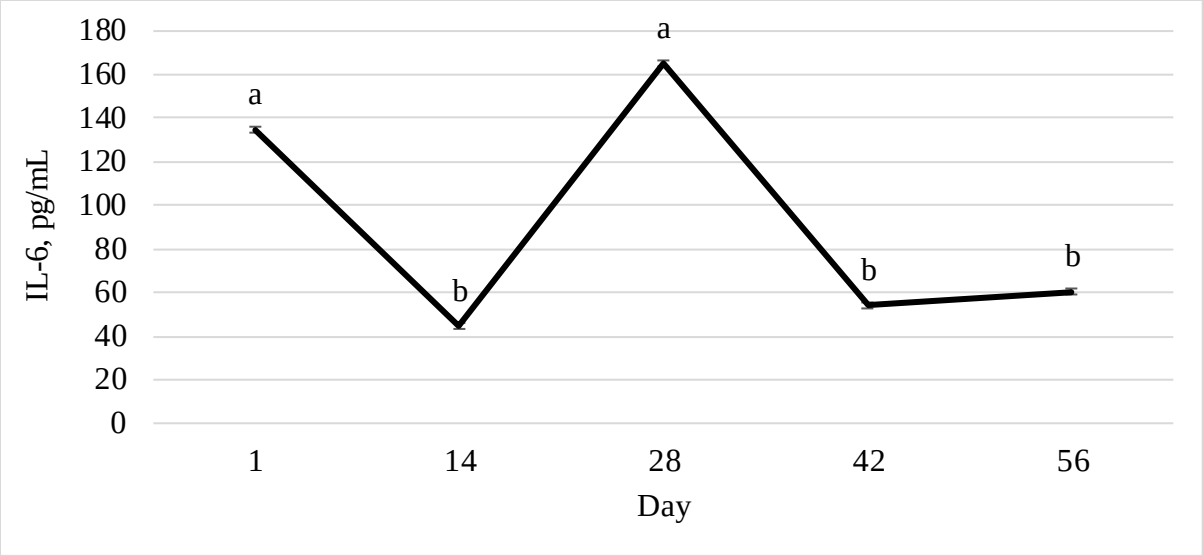


**Figure 4.1.** Average IL-6 concentration (pg/mL) of 42 newly received steers measured bi-weekly over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Different letters showing significant differences ($P < 0.05$) between days.

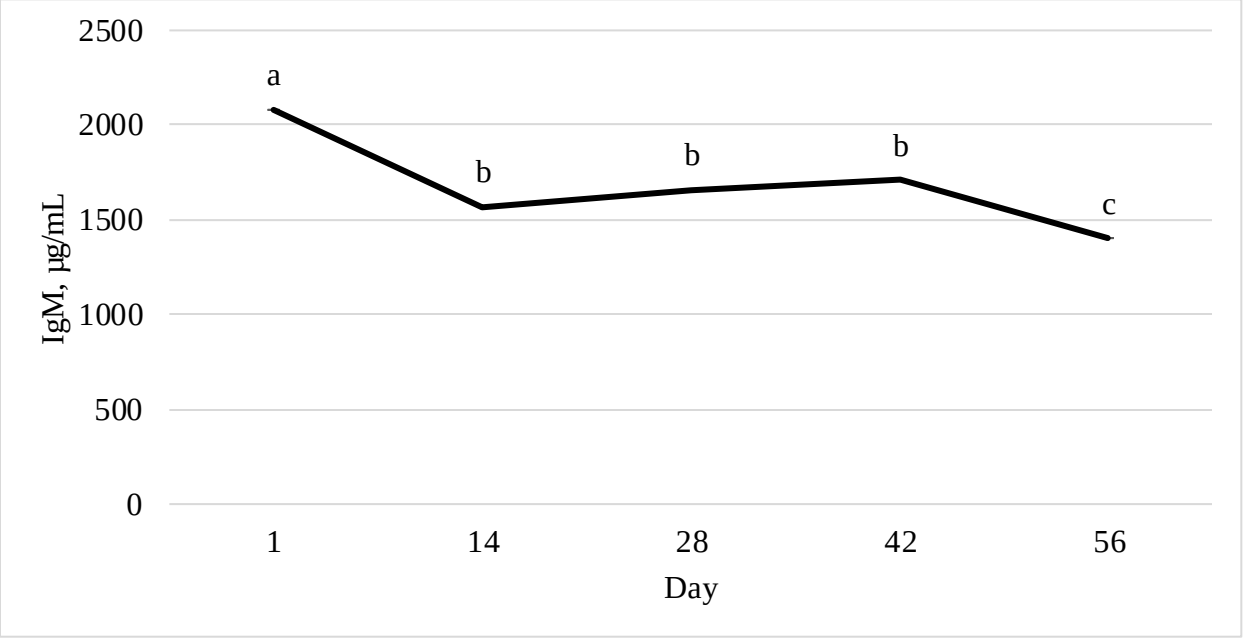


**Figure 4.2.** Average IgM concentration (µg/mL) of 42 newly received steers measured bi-weekly over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Different letters showing significant differences ($P < 0.05$) between days.

There was a significant treatment × day interaction ($P < 0.05$), where the haptoglobin concentration was greater in CT steers compared to SW and MX steers on d 14 (Figure 4.3). The concentration of serum amyloid A was higher ($P < 0.01$) on d 1 compared to the rest of sampling days (Figure 4.4). Fibrinogen concentration was greater ($P < 0.01$) on d 1 compared to d 14 (Figure 4.5).

The results from the white blood cell differential showed that the percentage of lymphocyte was greater ($P < 0.05$) on d 28 compared to other days (Figure 4.6), and the percentage of neutrophils was greater ($P < 0.01$) on d 56 than on d 1, 14 and 28 (Figure 4.7).

The concentration of cortisol in saliva was lower ($P < 0.01$; Table 4.2) on d 42 and d 56 compared to d 1 and 28 (Figure 4.8). The concentration of cortisol in hair was lower on d 56 compared to other days (Figure 4.9).

**Table 4.2**. Effect of flavouring additives on saliva and hair cortisol of newly received feedlot steers (n=42) during the 56-days feeding period.

| | Treatments[1] | | | | P-value | | |
|---|---|---|---|---|---|---|---|
| | CT | SW | MX | SEM[2] | Treatment | Day[3] | Treat × Day |
| Saliva cortisol, µg/dL | 0.12 | 0.13 | 0.12 | 0.236 | 0.32 | <0.01 | 0.55 |
| Hair cortisol, µg/dL | 0.08 | 0.08 | 0.08 | 0.133 | 0.45 | <0.01 | 0.24 |

[1] Data expressed as least square means. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/t (both provided by Lucta SA, Barcelona, Spain).

[2] Standard error of the mean.

[3] Saliva cortisol was measured on d 1, 14, 28, 42, and 56, while hair cortisol was measured on d 1, 28, and 56 of the study.

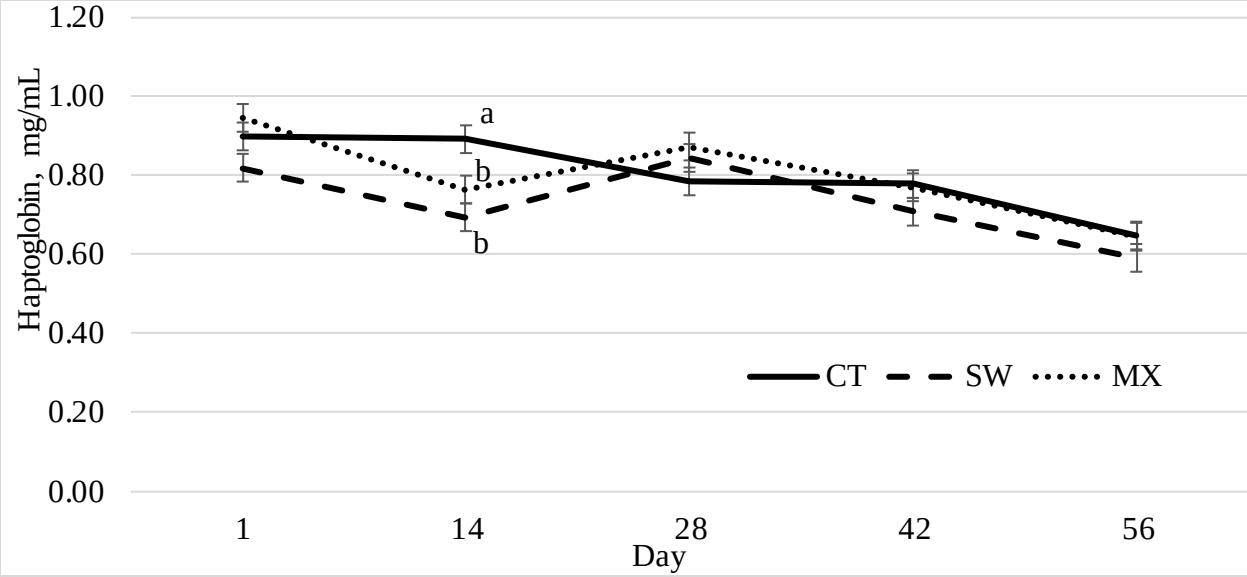


**Figure 4.3**. Average haptoglobin (mg/mL) of 42 newly received steers measured bi-weekly over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Treatments were: 1) standard feedlot receiving diet (control, CT); or the same diet with flavouring agents comprised of either sweeteners (SW) or a mix of basic tastes (MX) at 1 kg/Tn. Different letters showing significant differences ($P < 0.05$) among treatments within measuring periods.

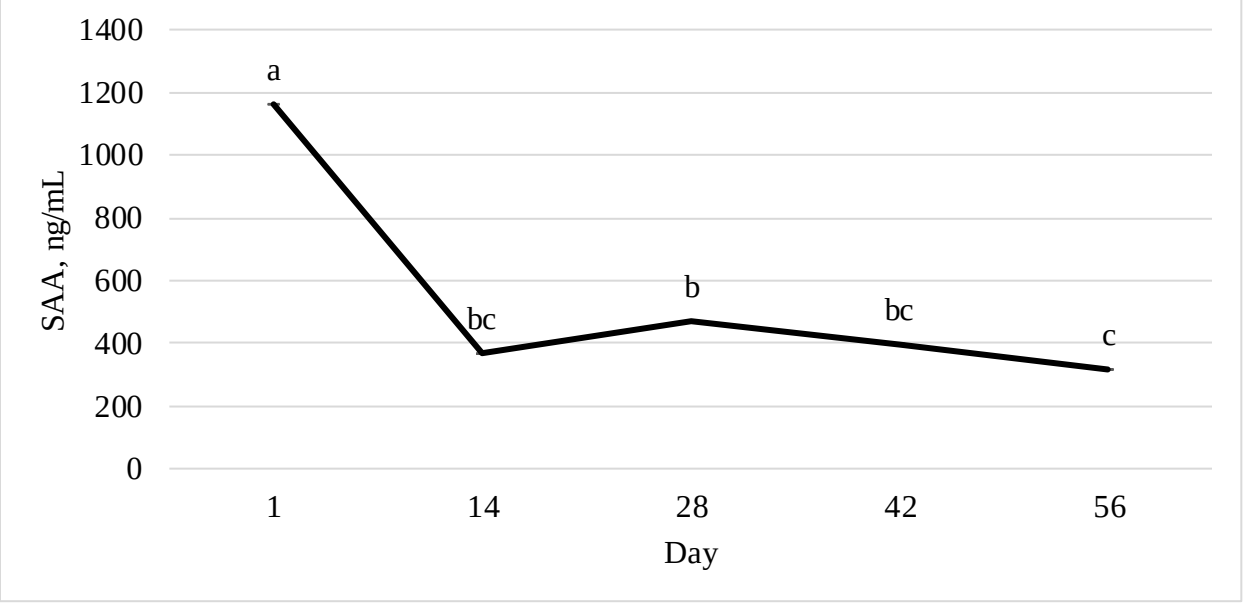


**Figure 4.4.** Average SAA concentration (ng/mL) of 42 newly received steers measured bi-weekly over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Different letters showing significant differences ($P < 0.05$) between days.

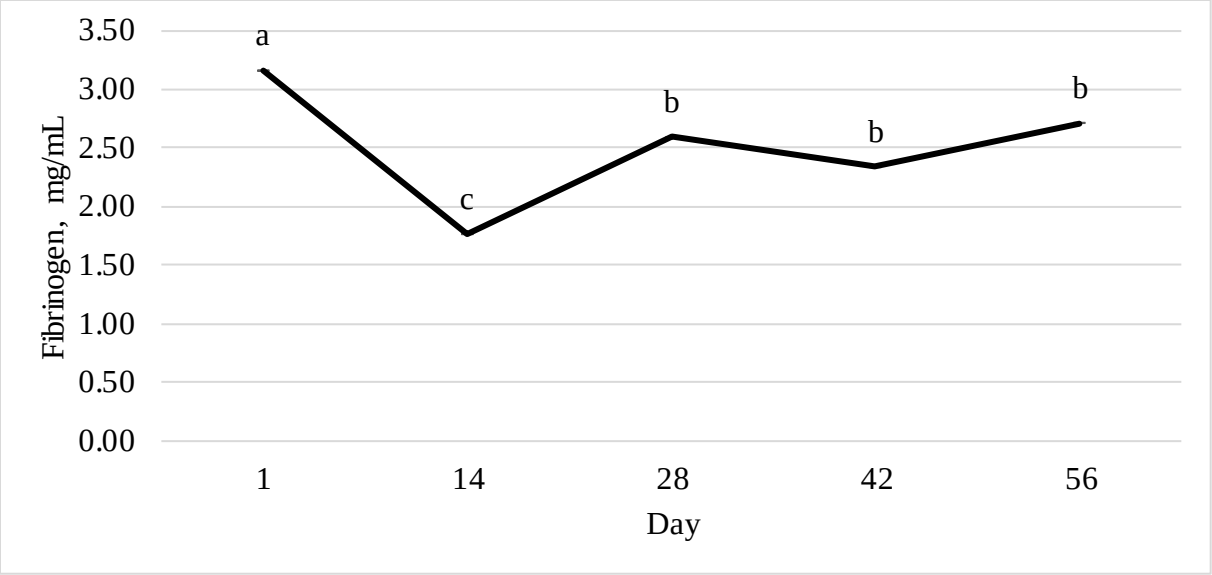


**Figure 4.5.** Average fibrinogen concentration (mg/mL) of 42 newly received steers measured bi-weekly over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Different letters showing significant differences ($P < 0.05$) between days.

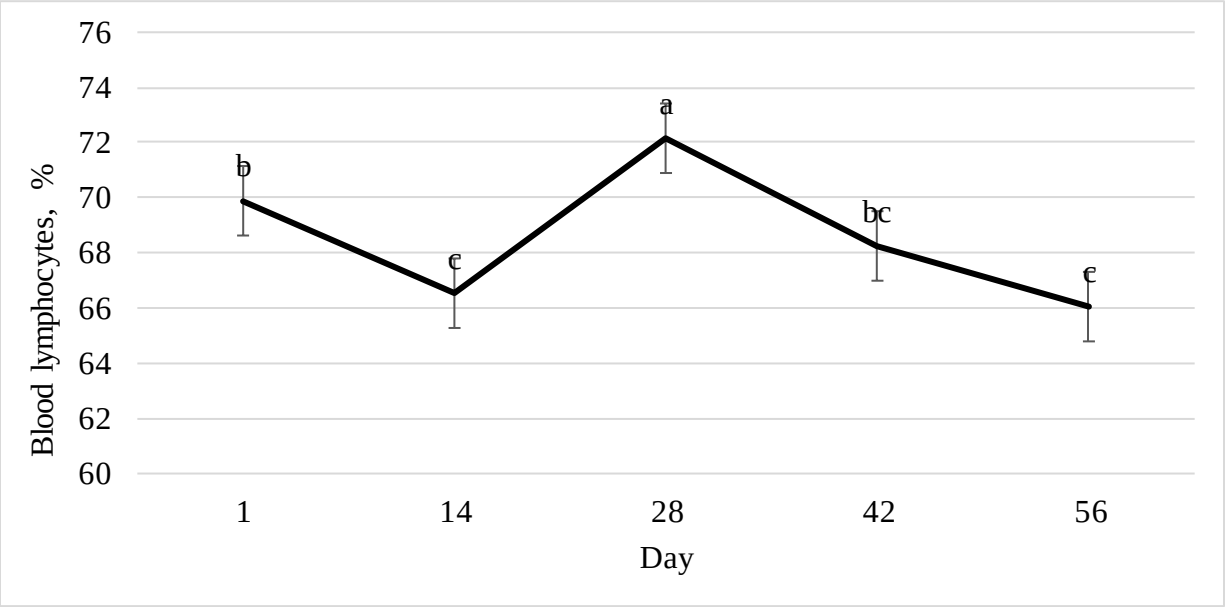


**Figure 4.6.** Average percentage of lymphocytes of 42 newly received steers measured bi-weekly over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Different letters showing significant differences ($P < 0.05$) between days.

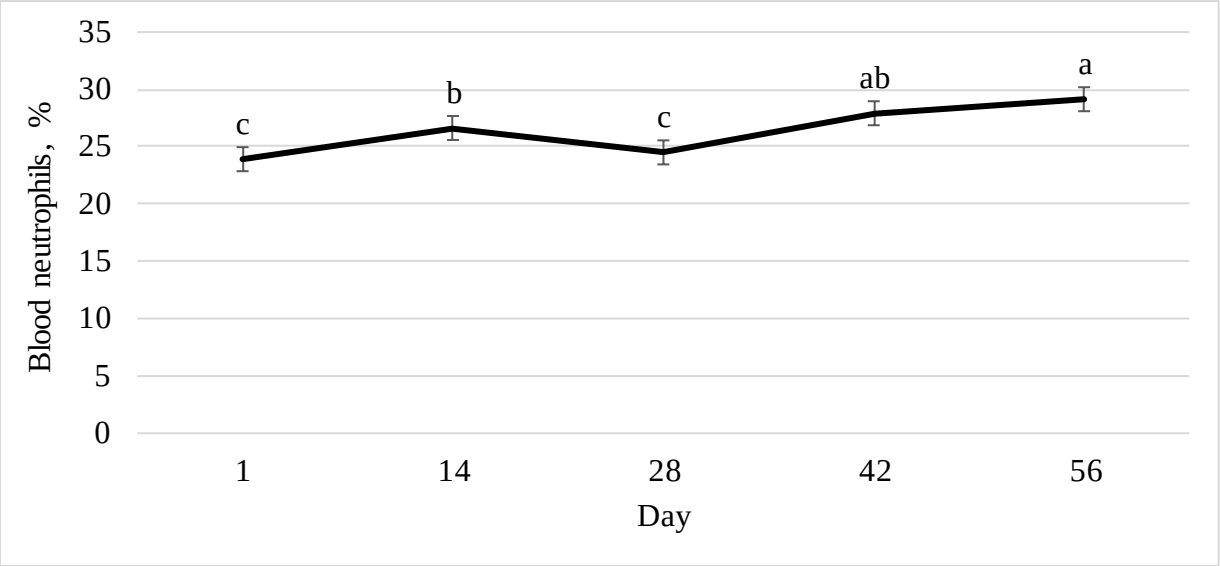


**Figure 4.7**. Average percentage of neutrophils of 42 newly received steers measured bi-weekly over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Different letters showing significant differences ($P < 0.05$) between days.

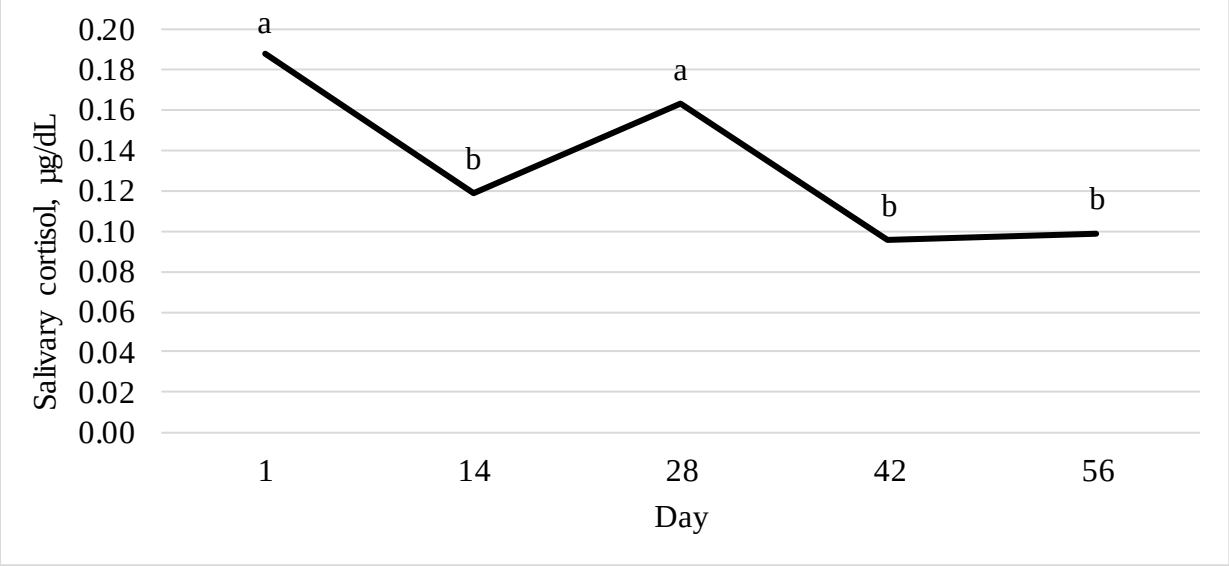


**Figure 4.8.** Average salivary cortisol (µg/dL) of 42 newly received steers measured bi-weekly over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Different letters showing significant differences ($P < 0.05$) between days.

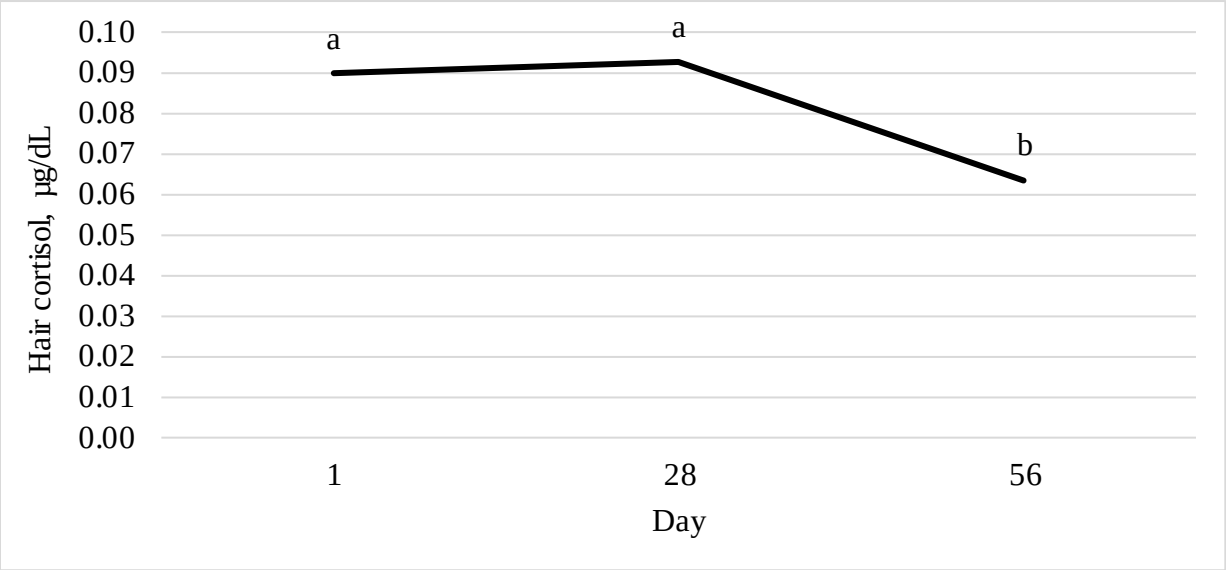


**Figure 4.9.** Average hair cortisol (µg/dL) of 42 newly received steers measured bi-weekly over the 56-d experiment. Data points represent LS means, while error bars are the SEM. Different letters showing significant differences ($P < 0.05$) between days.

### 4.4. Discussion

The concentration of blood acute phase proteins have been shown to increase following stress, disease, vaccination, or feed and water deprivation (Arthington et al., 2008; Marques et al., 2012; Cooke et al., 2013b). Arthington et al. (2005) also reported increased acute phase proteins in healthy calves upon weaning and transportation. This would explain why we found a greater concentration of IL-6, IgM, haptoglobin, and SAA on d 1, when have been recently weaned and transported, compared with d 56 of the study.

Based on the potential effects of flavouring additives at stimulating feed intake (Montoro et al., 2011) and changing the feeding pattern (Chapter 3 of this thesis) of cattle, we hypothesized that the use of those additives could prevent some of the negative consequences on the health and welfare of newly received feedlot cattle. Particularly those arising from the marked reduction in feed intake associated with weaning and transportation, and that may include an increased physiological stress (i.e. high cortisol levels) and an affected gastrointestinal tract functionality (i.e. compromised paracellular tissue permeability and mucosal and adaptive immune system).

However, we found no effects of our treatments on the biomarkers of inflammation and acute phase proteins, except for a reduced concentration of haptoglobin in SW compared to CT in d 14, which could be interpreted as a beneficial effect of sweeteners reducing the systemic inflammation of calves in response to the stressful challenge in the first weeks upon arriving to the feedlot. There is no clear explanation, however, on how SW could have caused this effect, as we found no biologically relevant effects of the treatments on the feeding pattern of steers during the first 3 weeks of the study (Chapter 3). This would suggest that either the effects of our treatments could not be detected under the circumstances of our study, with steers spending the first few weeks of the experiment adapting to the diet, feed bunk and pen mates; or that the effect of SW took place at a systemic level different to the hedonic response and the feeding pattern, and that we did not measure. Nevertheless, this effect on blood haptoglobin was not sustained over time and was not accompanied by any other changes in the rest of blood parameters measured. More research on this area is needed to confirm the relationship between positive hedonic response and the blunted release of acute phase proteins of calves under stressful situations. Yang et al. (2010) and De Souza et al. (2018) reported no effects on haptoglobin concentration with cinnamaldehyde supplementation in feedlot finishing cattle, or with a combination of sucram and plant extracts in newly weaned feedlot cattle, respectively. Xiaoping et al. (2020) reported no changes on IL-6 concentration and an increased IgM concentration in ewes provided with a mixture of plant extracts. However, differences between our results and those reported by them are likely due to different additives, diet, or animals used.

The proportion of neutrophil and lymphocytes observed in blood were similar to those described in the literature. Roland et al. (2014) described that the percentage of lymphocytes was around 80% in calves up to 3 months of age. Then, in adult cattle, the neutrophil to lymphocyte ratio was reduced to 1:2 according to Jones and Allison (2007) and Wood and Quiroz-Rocha (2010). In our study, with steers 4 to 7 months old, the average neutrophil to lymphocyte ratio was 1:2.6. Besides the age effect, the low number of neutrophils observed at the beginning of our study could be linked to the suppressing effect on an increased concentration of inflammatory mediators.

In the transfer from cow-calf operations to feedlots, calves go under multiple stress challenges resulting in the previously described increased plasma concentrations of pro-inflammatory cytokines and acute phase proteins (Jaffer et al., 2010; Cooke, 2017). This is in

agreement with the increased concentration of salivary and hair cortisol we found at the beginning of our study, likely due to the effects of the stress associated with weaning (Hernandez et al., 2014), transportation (Burdick et al., 2011a), and change of diet and environment (Bourguet et al., 2011). The steady level of hair cortisol on d 1 and 28 compared to d 56 suggests are likely reflecting the stress faced during the processing and in the acclimation period for the first few weeks of the study. The salivary cortisol concentration showed a steady decline (with an increased on d 28, likely due to subclinical infection, which could not detect) throughout the experiment, suggesting habituation to the repeated handling and the environment (Andrade et al., 2001).

The flavouring additives in this study showed no effects on the white blood cell differential on newly received feedlot steers. In agreement with this, De Souza et al. (2018), McMeniman et al. (2006), and Ponce et al. (2014) also reported no effects of sweeteners or plant based extracts on the immune function of cattle, suggesting that this approach may not have any effect on the incidence or severity of diseases in receiving cattle.

### 4.5. Conclusions

Changes observed in the biomarkers of stress, inflammation and immune function are compatible with the stress and challenges experienced by receiving steers at the beginning of the trial. The reduced cortisol concentration at the end of the study suggests the steers were acclimated with the new environment and diet. Under the conditions of our study, the addition of flavouring agents did not show a significant effect on the concentration of acute phase proteins, inflammatory mediators, cortisol or the percentage of neutrophil and lymphocytes. A different combination of flavouring agents, dose, or strategy to use flavouring agents (one flavour profile vs. a rotation of flavours over time) should be explored in future research trials to further assess the efficacy of these additives in newly received feedlot cattle.

# 5. General discussions and conclusions

Under the conditions of our study, the use of flavouring additives, and mainly the one based on sweet components (SW), had a modest effect on the performance of receiving feedlot cattle, supported by an increase of the ADG and feed efficiency only during specific periods of the experiment. Sweeteners can be added into the diets through incorporation into feed by mixing machine or as a top dressing to attract animals to visit the feed bunk. The increased effects for the growth performance that have been found here, however, were not significant when considering the performance and efficiency of the steers over the whole 56-feeding period, suggesting that those significant effects could be due to either an artifact of the measurements (BW can be highly variable depending on the gut filling of the steers at the specific moment of weighing), or rather that those differences were biologically relevant, but there was some compensatory growth between treatment groups that masked the effects over the length of the study. Looking at the coefficient of variance of the BW when this was measured over two consecutive days, we found that BW variability was quite low (2.3% in d 1 and 2, and 1.5% in d 56 and 57 of the study). This would suggest that single-day weights on d 14, 28 and 42 may have been reliable, hence the effects on growth performance are more likely explain due to BW fluctuations in response to treatment effects and compensatory growths.

Similarly, we found several effects of the flavouring additives (and again, mostly in the treatment comprised of sweet components) on the feeding pattern of the steers, but they were all transient in nature, with no clear biologically relevant trends. Around weeks 4 and 5 of the study, SW steers were eating faster and making more frequent but shorter and smaller visits to the feed bunk. This increased frequency of feeding events is considered to be a desirable behaviour to optimize DMI, as it warrants a more constant provision of energy to the rumen microbiota throughout the day, and attenuates the variability of rumen pH, minimizing the risk of developing digestive upsets (e.g., acidosis) (Gonzalez et al., 2008; Moya et al., 2011). The reason why SW effects on feeding pattern peaked on wk 4 could be that either the greater data variability associated with steers adapting to the diet, feed bunks and pen mates masked the treatment effects during wk 1 to 3, or that cattle needed the exposure to SW over a few weeks for them to change their feeding pattern. Previous studies have also reported a delayed response of beef cattle to flavouring

additives, such as changes on DMI from d 21 to 56 (Ponce et al., 2004), or changes on the ADG from d 28 to 56 (McMeniman et al., 2006).

In this study, these effects on the feeding pattern were only reflected in a greater DMI on wk 6, which matched the greater ADG and feed efficiency that took place around the same time of the experiment. This suggests that the changes on the feeding pattern did not have the goal of ingesting more feed, but they were rather the result of the different behavioural response to the feed offered, likely driven by the hedonic response associated to the different treatments. The lack of consistency over the 56-d study, however, may indicate that steers may have got used to such oro-sensory stimulus over time (Villalba et al., 2011), or that other factors (e.g., competition at the feed bunk, development of social hierarchies, boredom, or habituation to the diet) influenced their behaviours and choices at the feed bunk (Dalton and Finlayson, 2013). Further research is necessary to better understand the role of flavouring additives in the feeding pattern of cattle.

We hypothesized that a positive change in the hedonic response of cattle to the diet would stimulate feed intake and reduce the stress caused associated with the receiving period of feedlot calves. This would have an impact on their temperament, and more calm reactions to handling could be observed over the length of the study. In this study, however, we were not able to detect any major effect in the flight speed or chute behaviour of steers. The trend for a less abrupt range of movement at the chute in SW compared to CT steers could be contributing to the increased ADG during d 1-14 and 28-41, as calmer temperaments have been associated with better performances (Bruno et al., 2016). In our study, however, the association between lower movements at the chute and calm temperaments was not further supported with other results, such as with different levels of saliva or hair cortisol (Haskell et al., 2014). It could be that the specific tests we used were not good enough at characterizing the wide spectrum of temperament traits of beef steers (Réale et al., 2007), as both tests were run simultaneously as steers were being processed and sampled at the chute, which is quite a novel and stressful experience for these animals, and hence could be masking subtle changes of temperament. In this study, we purposedly wanted to limit the amount of handling of the steers, as we did not want to interfere with what a regular receiving process would be in a commercial setting. More research could be done in the future to implement more tests (e.g., novel object test, study of pen behaviour, open-field test,

judgment bias test) to further scrutinize the social, exploratory, boldness traits of these animals, rather than focus only on the fear reactivity to human handling.

The results obtained on the immune function and biomarkers of inflammation and stress showed that steers were at a higher level of stress and with the acute phase response activated upon entry to the feedlot compared to the end of the study, likely due to the natural adaptation of the steers to the diet and environment (Marques et al., 2012; Cooke et al., 2013b). Under the conditions of our study, we found no effects of adding flavouring additives on the different biomarkers of stress, inflammation, or immune function, except a reduced concentration of haptoglobin in SW compared to CT in d 14. Although this effect was not consistent over time, it could be indicative of a potential effect of SW at a systemic level, which could have contributed to the improved ADG and FE of SW steers during specific periods of the study, as Arthington et al. (2005) reported a negative association between haptoglobin concentration and ADG of steers.

This, along with the changes in the feeding pattern and chute behaviour, could be interpreted as a beneficial effect of sweeteners reducing the physiological response of calves to the stressful challenge in the first weeks upon arriving to the feedlot. The lack of consistency over time, and the lack of biological repercussion in any of the other parameters analyzed, limits the interpretation of such effect. More research on this area is needed to confirm the relationship between positive hedonic response and what could have been a blunted release of acute phase proteins of calves under stressful situations.

In conclusion, under the conditions of our study, and based on the results exposed in Chapters 3 and 4, the use of flavouring agents did not have a clear positive impact on the performance, health or welfare of newly arrived feedlot cattle. Future research could further assess the efficacy of these additives in newly received feedlot cattle using a different combination of flavouring agents; evaluating possible interactions between diet composition, feeding management and the efficacy of such additives; exploring different levels of inclusion; or assessing different strategies to use such flavouring additives (is one flavour profile stimulating enough for the calves over time, or do we need expose cattle to different flavours over time to prevent the aforementioned sensory-specific satiety effect).

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