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Late weaning improves growth performance and rumen development in Alpine goats

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Résumé

La présente étude visait à déterminer les effets de l'âge de sevrage sur les performances de croissance, le développement du rumen et le microbiote chez les chevreaux alpins. Soixante-douze chevreaux ont été assignés au hasard par paires mâle et femelle à l'un des trois traitements. 1) sevrage précoce (EW), à l'âge de 6 semaines, 2) sevrage moyen (MW), à l'âge de 8 semaines (MW) et 3) sevrage tardif (LW), à 10 semaines d'âge (LW). Le lait de remplacement a été proposé *ad libitum* jusqu'à la semaine de sevrage, où le lait a été réduit de 12.5 % par jour pendant sept jours. Deux semaines après la naissance, du concentré, du foin et de l'eau ont été offerts à volonté jusqu'à l'âge de 12 semaines où les chevreaux ont été abattus. La consommation a été enregistrée quotidiennement et le poids corporel (PC) a été enregistré chaque semaine. Pour évaluer le développement du rumen, des échantillons de sang ont été prélevés pendant tout l'essai et analysés pour le β-hydroxybutyrate sanguin (BHB) et les acides gras non estérifiés (NEFA). Les mesures ruminales ont été prises à la semaine 12 avec les trente-six mâles seulement. Le contenu du rumen a été obtenu pour l'analyse de la composition bactérienne en utilisant la région V4 du gène de l'ARNr 16S et la qPCR a été utilisée pour quantifier les bactéries, les protozoaires et les champignons. Les papilles du rumen ont été analysées dans 4 régions du rumen : atrium du rumen (RA), sac ventral (VS), Cul-de-sac caudo-dorsal (DS) et Cul-de-sac caudo-ventral (VBS). Les chevreaux sevrés à 10 semaines, contre 8 semaines et 6 semaines avaient un gain quotidien moyen plus élevé pour la semaine post-sevrage (0.35 vs. 0.24 vs. 0.24 kg/j) et étaient plus lourds à la 12e semaine (27.44 vs. 25.45 vs. 24.07 kg, $P < .05$). Chez les animaux LW, le PC n'a pas été affecté pendant la période post-sevrage; probablement en raison d'un apport élevé en énergie métabolisable (EM) causé par la stratégie de sevrage contrairement à ce que nous observons chez les animaux EW et MW. Les taux sanguins de BHB ont augmenté au moment du sevrage pour tous les traitements, mais étaient plus élevés chez les chevreaux EW par rapport aux chevreaux MW (+ 21 %) et LW (+ 41 %) ($P < .05$). Les taux sanguins de NEFA chez les chevreaux EW ont augmenté au sevrage et étaient plus élevés que MW (+ 40 %) et LW (+ 101 %) chez les chevreaux ($P < .05$), suggérant une mobilisation plus prononcée du tissu adipeux chez les chevreaux sevrés précocement au sevrage. La population de microbiote en post-sevrage a montré que si le sevrage était retardé, l'abondance des bactéries totales semblait augmenter ($P < .05$) par rapport aux

animaux EW, tandis que les protozoaires et les champignons diminuaient pour les LW. L'âge du sevrage semble induire des modifications du microbiote ruminal au cours du post-sevrage. Les mesures d'absorptiométrie à rayons X à double énergie (DEXA) des carcasses ont été utilisées comme indication de la récupération de la composition des graisses après le sevrage. Le tissu adipeux (%) dans LW était (9.4%) et EW était (7.2%) plus élevé par rapport à MW (P< .05). La surface totale de la papille était plus grande chez les chevreaux LW, par rapport aux chevreaux MW (+ 49 %) et EW (+ 22 %) (P< .05). Globalement, le sevrage des chevreaux à l'âge de 10 semaines a limité l'impact négatif d'un sevrage précoce sur la croissance et le développement du rumen de la race alpine.

Mots-clés : Chevreaux, sevrage, développement du rumen, performances de croissance

Abstract

The present study aimed to determine the effects of weaning age on growth performance, rumen development and microbiota composition in Alpine goat kids. Seventy-two kids were randomly assigned in pairs male and female to one of three treatments. 1) early weaning, at 6 wk of age (EW), 2) medium weaning, at 8 wk of age (MW) and 3) late weaning, at 10 wk of age (LW). Milk replacer (MR) was offered *ad libitum* until the step-down wk, when milk was reduced by 12.5% per day for seven days. Two wk after birth, starter ration, hay, and water were offered *ad libitum* until 12 wk of age where kids were slaughtered. Feed intake was recorded daily, and body weight (BW) was recorded weekly. To evaluate rumen development, blood samples were taken during the whole trial and analyzed for blood β-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA). Ruminal measurements such as papillae, VFAs and microbiota were taken at wk 12 with only thirty-six males. Rumen contents were obtained for bacteria composition analysis using the V4 region of the 16S rRNA gene and qPCR was used to quantify bacteria, protozoa and fungi. Rumen papillae were obtained for histological analysis in 4 rumen areas: rumen atrium (RA), ventral sac (VS), caudodorsal blind sac (DS), and caudoventral blind sac (VBS). Kids weaned at 10 wk, compared with 8 wk and 6wk had higher average daily gain for the wk postweaning (0.35 vs. 0.24 vs. 0.24 kg / d) and were heavier at wk 12 (27.44 vs. 25.45 vs. 24.07 kg, $P < .05$). In LW animals, BW was not affected during post weaning period; possibly due to high metabolizable energy (ME) intake caused by the weaning strategy contrary to what was observed in EW and MW animals. Blood levels of BHB increased at weaning time for all treatments but were higher in EW compared to MW (+21%) and LW (+41%) kids ($P < .05$). Blood levels of NEFAs in EW kids spiked at weaning and were higher than MW (+ 40%) and LW (+101%) in kids ($P < .05$), suggesting a more pronounced adipose tissue mobilization in early weaned kids at weaning. Microbiota population in postweaning showed that while weaning was delayed, abundance of total bacteria seemed to increase ($P < .05$) compared with EW animals, whereas protozoa and fungi decreased for LW. Weaning age influences the ruminal microbiota during postweaning. Dual-energy X-ray absorptiometry (DEXA) measurements of carcasses were used as an indication of fat composition recovery in post-weaning. Fat tissue (%) in LW was (9.4%), and EW was (7.2%) higher compared to MW ($P < .05$). The total papilla surface area was greater in LW, compared to MW (+49%) and

EW (+22%) kids (*P*< .05). Overall weaning kids at 10 wk of age limited the negative impact of earlier weaning on growth and rumen development in the Alpine breed.

Key words: Goat kids, weaning, rumen development, growth performance.

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List of Acronyms and Abbreviations

ADG: Average Daily Gain

AE: anion exchanger

BHB: β-hydroxybutyrate

BW: Body Weight

C2: Acetic acid

C3: Propionic acid

CDK2: Cyclin-dependent kinase 2

CH_4: Methane

CO_2: Carbon dioxide

CP: Crude Protein

DMI: Dry Matter Intake

DS: Caudodorsal blind sac

EW: Early weaning

GIT: Gastrointestinal tract

GPR41: Protein-coupled receptors 41

H^+: Cationic form of atomic hydrogen

Kg: Kilograms

LPS: Lipopolysaccharide

LW: Late weaning

MCT: Monocarboxylate cotransporter isoforms

ME: Metabolic Energy

MR: Milk replacer

MW: Medium weaning

Na^+ / K^+ -ATPases: Sodium-potassium pump

NBC: Sodium/bicarbonate Cotransporter

NH_3: Ammonia

NHE: Sodium/proton exchanger

Ph: Power of hydrogen

pKa: Acid dissociation constant

RA: Rumen atrium

VBS: Caudoventral blind sac

VFAs: Volatile Fatty Acids

VS: Ventral sac

WK: weeks

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Introduction

The success of any replacement program in dairy goat production depends on raising and developing animals that reach an optimal size and weight to initiate puberty, breed easily at an appropriate age and at the lowest possible cost (Lu and Potchoiba, 1988). The digestive system of ruminants undergoes changes between birth and the moment in which the rumen becomes functional. Nutrient absorption changes are observed during the transition from milk to solid feed. Such dietary change alters the pattern of nutrients delivered to the intestine and the liver, and thus to the peripheral tissues of the animal (Baldwin et al., 2004). The dramatic shift at weaning, when milk replacer is completely removed, triggers rumen microbiota shifts and fermentation process. Resulting from ruminal microbial fermentation, volatile fatty acids (VFAs) are produced and then stimulate ruminal papillae development, muscular development, and expansion of ruminal volume development, providing niche environments for microbiota able to process highly fibrous feedstuff (Weary and Keyserlingk, 2011; Meale et al., 2017). Butyric acid, which is transformed into β-hydroxybutyrate (BHB) in the rumen epithelium, is one of the major end products of bacterial carbohydrate fermentation and is recognized as a molecule that regulates differentiation and proliferation of the rumen tissue (Baldwin and Connor, 2017), which is in turn necessary for optimal absorption of VFAs. Weaning stress can reduce feed intake and consequently negatively impact growth. Importantly, the age at which the animal is weaned can modulate these effects, possibly due to better ruminal and intestinal development (Eckert et al., 2015). However, to our knowledge, there are no published studies that established optimal weaning age based on growth performance, histomorphology, and microbial shifts in the rumen of goat kids. For this reason, the objectives of this study were to: 1) Evaluate the effect of weaning at 6 (EW), 8 (MW) and 10 (LW) weeks of age on growth performance and rumen development, and 2) Characterize the differences in ruminal microbiota occurring as a result of different weaning ages. We hypothesized that late weaning age improves growth performance, rumen development and ruminal microbiota in kids fed with milk replacer.

Chapter 1 – Literature Review

1.1 Dairy goat production

Goat milk represented 1.9% of the total milk production in the world around (799 Mt) in 2016, Cattle (83.1%) and buffaloes (13.1%) are the main milk producing animals in the world (Pulina et al., 2018). The continents with the highest goat farming according to Pulina et al., (2018) are Asia (52.1%) and Africa (39.6%), then Europe (4.3%), the Americas (4.0%), and Oceania (<0.1%). In North America, the United States is the country with the most herds of goats, estimated at 2.6 million heads in 2018, followed by Canada with 230 034 heads (Lu and Miller, 2019). Goat industry in Canada is divided into three sectors: meat production, milk production and fiber such as mohair and cashmere(CDIC, 2021) with a total of 136 876 herds that are specialized farms. Dairy goat industry has been growing progressively in Canada, going from 22.1 million liters in 2007, to 62.2 million liters in 2018 (CDIC, 2019), with most of the production located in Ontario followed by Quebec and Alberta (CDIC, 2018). Goat's milk has become important due to its functional properties and nutritional value (Verruck et al., 2019), in addition to its benefits in human health compared to cow's milk. Research in dairy goat products has been focused on the chemical composition, which is rich in proteins of high biological value, essential fatty acids (Kompan and Komprej, 2012), high mineral bioavailability and vitamin content make dairy goats products of economic relevance in the market (Clark and García, 2017)

1.2 Dairy goat production in Quebec

Quebec's goat milk sector is the second largest in Canada in terms of goat milk production and manufacturing of milk products. However, the sector is experiencing a decrease in its market share to the detriment of Ontario, which is experiencing increased growth (CDIC, 2018) Production in Quebec has become stagnant, and the sector faces a series of challenges, including lack of qualified labor, farm growth, lack of improved genetics, and decline of milk quality (MAPAQ, 2017). The main costs of commercial dairy goat farms are feed, labor, and debt interest costs (Lu and Miller, 2019). The pressure on Quebec goat producers increased sharply in 2018,

with several Quebec processors threatening to stop their purchases in Quebec. Faced with this pressure, dairy goat companies in Quebec are dealing with financial insecurity and trying to identify solutions for economic improvement (CECPA, 2020). In Quebec, the cost of producing one hectolitre of goat milk varies enormously (155 to 242 $ / hl). The main culprits for this variation are the productivity of the goats, the cost of feed and labor time (CECPA, 2020). Like dairy cow industry, profitability of goat milk production depends on its production efficiency, which can be improved by implementing better management and feeding practices. In Quebec Goat milk is mainly used for cheese production which highlights the importance of producing high quality milk in terms of milk components (i.e. protein and fat) and somatic cells. It appears that efforts in better technologies, and management practices in goats are convenient to produce quality milk since dairy goat takes economic importance in the province.

1.3 The ruminant animal

Ruminant gastrointestinal trac exhibits a series of anatomical adaptations determined by the type of feed available through their evolutionary history (Van Soest, 1994). In addition to the capacity of postgastric fermentation observed in other herbivores (e.g. the horse), ruminant has a large pregastric organ complex colonized by diverse microbial populations, which degrade fibrous feeds prior to reaching the true stomach (i.e. the abomasum), where they are subjected to both enzymatic and acid degradation (Czerkawski, 1986). Ruminant gastric complex is a multicompartment organ characterized by a marked expansion of the esophageal region divided into three distinct and voluminous pregastric diverticula: rumen, reticulum, and omasum; with a final glandular portion capable of enzyme and acid secretion, the abomasum (Fails and Magee, 2003). The complete gastric complex occupies approximately three-quarters of the abdominal cavity and it is placed on the left half, and it extends over the median plane to the right (Van Soest, 1994). The rumen has an average volumetric capacity of 15 - 22 L in small ruminants (Millen et al., 2018), where various chemical reactions catalyzed by microbial enzymes take place. In addition, the rumen is characterized by the presence of thick muscular bands called pillars, which divide the rumen into sacs and help in the mixing of feeds within it; the special anatomy on the rumen has an importance to allow the free passage of feed between compartments and stimulate

fermentation (Millen et al., 2018). Indeed, rumen musculature allows for contractions and good mixing of the digesta with the microbiota, due to an equilibrate mechanism of transfer, suspensions, and residence time of particles having a minimum of stagnation within the rumen, and ensures microbial action of the enzymes on the fiber (Czerkawski, 1986). Ruminal tissue tends to be keratinized and differs from other skin surfaces by its electrical resistance, permeability, and transport characteristics, which includes abundant and voluminous papillae necessary for the absorption of volatile fatty acids (VFAs) (Van Soest, 1994). The intensive microbial degradation of fibrous feedstuffs composed of carbohydrate polymers, entails their hydrolyzation to small saccharides, which in turn, are fermented to numerous products, mainly VFAs, lactic acid, CO_2, CH_4, and ammonia (NH_3). The proportions of these products can change with diet type and feeding intensity, causing changes in microbial population composition and metabolism (Russell and Hespell, 1981).

VFAs and NH_3 are absorbed at the epithelium of rumen walls (Abdoun et al., 2006). Ruminants release CH_4 and CO_2 through eructation, or by transport via circulation to the lungs to be exhaled. (Hill et al., 2016). The rest of the fermentation products, and part of the microbial mass, pass to the abomasum, where they are digested by host enzymes. Once feed particles have been reduced in size by chewing and fermentation, feedstuffs pass into the omasum. This area has the appearance of an open book with tissue likened to the pages of a book, called leaves. These leaves have small papillae which absorb a large portion of the VFAs, water, and electrolytes such as potassium and sodium that were not absorbed through the rumen wall drying out feed particles until they reach the next compartment (Umphrey, 1992).

1.4 Morphological rumen development

Embryonic developmental stages of the ruminant stomach extend from its early fusiform stage to its final configuration during the first 55 to 60 days (Figure 1) (Kalenberg and Stoffel, 2020). This process occurs into three stages: in the first one there is no compartmental differentiation; in the second one, the rumino-reticulum, omasum and abomasum have differentiated; and in the third one, every compartment is differentiated (Vivo et al., 1990).

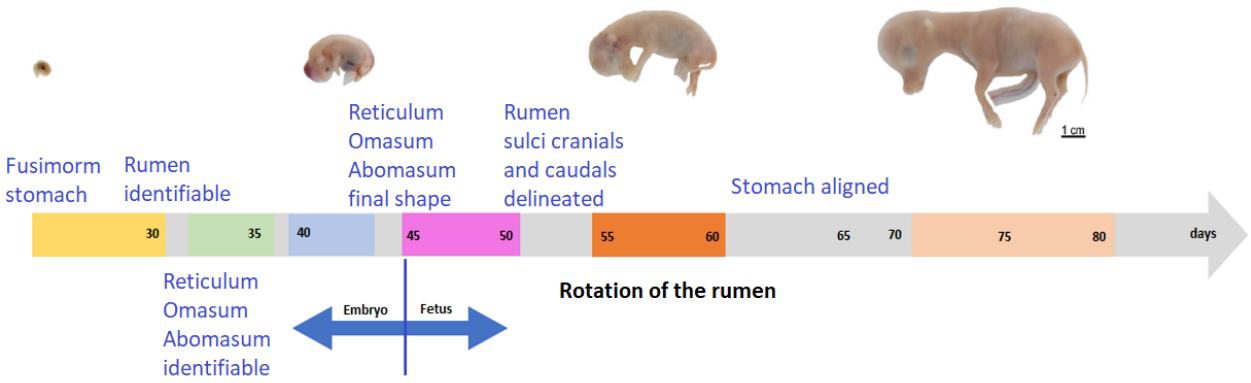

Figure 1. Chronology of the bovine gastric complex development. Colored bars represent developmental processes, vertical lines indicate the occurrence of corresponding features (an adaptation of Kalenberg and Stoffel, 2020).

From birth until the moment in which the rumen becomes functional, the digestive system undergoes many changes. The rumen tissue in the neonate is thin, without papillae or musculature and does not have any degree of keratinization (Baldwin et al., 2004). Prior to weaning of young nursing ruminants, the entrance to the rumen is closed by the esophageal groove reflex, a muscular canal that closes allowing liquid to bypass the rumen. Fluid milk flow down directly into the omasum, and this event is stimulated by suckling (Dehority, 2002). During the pre-ruminant phase, nutrients from milk or milk replacers are digested by the animal's gastric and intestinal enzymes, which allow for the degradation of milk proteins, lactose, and dietary lipids (Drackley, 2008). During the transition from liquid to solid feed, the young ruminant starts its gastro intestinal increases in rumen mass, papillae development, and metabolical development (Baldwin et al., 2004). The development of muscle mass in the rumen is stimulated by feed, but the development of the epithelium is directly related to the fermentation carried out by microbial organisms that colonize the rumen specifically by diet containing solid feed (Xie et al., 2013). Rumen tissue development then is important to maintain a fermentation chamber with an appropriate environment for absorptive functions, and protection of microbial infiltration. The ruminal epithelium (Figure 2) is a squamous and stratified tissue with different four layers: stratum basal, stratum spinosum, stratum granulosum, and stratum corneum, plus an outer layer of keratin and this ensemble is called the epidermis (Steele et al., 2016; Yohe et al., 2016).

RUMINAL EPITHELIUM

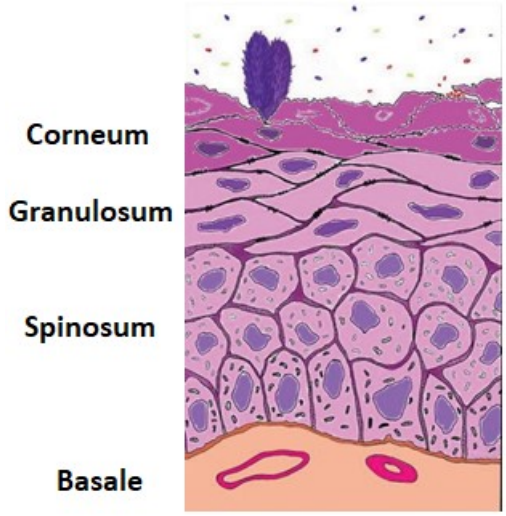

Figure 2. Ruminal epithelium, stratified squamous epithelium of the rumen an adaptation of Steele et al., (2016)

The stratum basale, and the stratum spinosum, are a composed of cells that contain fully functional mitochondria and other organelles to generated mitochondrial-ATP to be used by Na+ / K+ -ATPases and to generate the asymmetric ion gradients that energize polarised secondary active absorption and secretion by epithelial tissue (Graham and Simmons, 2005). The cells of these layers contribute to most of the metabolic properties related to whole-energy metabolism in the ruminant (i.e., ketogenesis) (Vi, 1998). The stratum granulosum, where the expression of occluding junctions complex is carried out, acts as a barrier against the passive paracellular entry of substances from the ruminal lumen (Meissner et al., 2017; Stumpff et al., 2011). The stratum corneum is a keratin sheet formed by layers of cells consisting mainly of a filament-amorphous matrix complex enveloped by a thickened plasma membrane that form cornified keratinocytes call horny cell (Lavker and Matoltsy, 1970) and is in contact with the ruminal content, the corneum surface acts as a physical barrier protecting the lower layers (Steele et al., 2011) and is colonized by microbes that are unable to migrate towards the stratum granulosum. (Steele et al., 2016)

1.5 Milk feeding phase

The milk feeding phase represents the period in which animals consume milk replacer until the time when animals can consume amounts of solid feed enough to fulfill their total energy requirements (Lu and Potchoiba, 1988). Milk replacer components (i.e. fat, lactose, and protein) are digested in the abomasum by abomasal enzymes (e.g. pregastric lipase, rennin, and pepsin) and then by enzymatic activity in the intestine (e.g. pancreatic lipase, trypsin, chymotrypsin, carboxypeptidase, elastase, and lactase). The result of this digestion will provide small- and medium-chain fatty acids, glucose, galactose, and amino acids for the maintenance of vital body functions and growth (Khan et al., 2011). Milk is the only source of energy for newborns, currently, artificial rearing becomes relevant, and the type of milk fed can be either whole milk, waste milk and reconstituted milk replacer. These types of milk are a profitable option that allows raise newborns with a nutritionally balanced product and producers increase the quantity of milk of the doe available to sell. Generally, new ruminants are fed milk twice daily from a nipple bottle, bucket, or an open bucket (Hammell et al., 1988; Amaral-Phillips et al., 2006). During milk feeding phase, the esophageal groove reflex closure, bypass the rumen (Ørskov et al., 1970) to allow milk directly enters into the abomasum to be used by the animal. This reflex is stimulated by visual, auditory, suction; and oral receptors (Ivany et al., 2002) delivering essential nutrients to the abomasum avoiding potential fermentative breakdown in the rumen.

Intake in early life (milk replacer, starter, hay) is an important factor that determines growth rate. High starter and milk replacer intake during preweaning balances energy requirements during the weaning period and postweaning. (Sampelayo et al., 1990; Yeom et al., 2002). Studies recommend (Bartlett et al., 2006) milk replacers with around 26% and 28% of CP to have higher weight gain. In preweaning, the milk quality, in terms of crude protein, is directly related to increases in dry matter consumption (DMI) and growth performance. Goat kids have a lower voluntary feed intake (Hadjipanayiotou and Sampelayo, 1997) therefore, growth performance in early life can be explained by the quality of the protein in the diet (Lu and Potchoiba, 1990; Firoozi et al., 2017). Milk replacers contain two types of protein, casein and whey proteins which include β-lactoglobulin (β-LG, for short), α-lactalbumin (α-LA), immunoglobulins (IG), bovine serum albumin (BSA), bovine lactoferrin (BLF) and lactoperoxidase (LP) (Madureira et al., 2007). Whey is

broadly used because it quickly dissociates for passage to the small intestine and this source is much more digestible for the animal (Erickson and Kalscheur, 2020). In young ruminants, milk withdrawal encourages consumption of feeds with a high starch content, which promotes ruminal papillae development, as well as hay, which increases ruminal mass (Baldwin and Connor, 2017; Baldwin et al., 2004). However, this transition causes strong behaviours indicative of stress such as vocalization (Budzynska and Weary, 2008; Magistrelli et al., 2013) and low ADG (Khan et al., 2007; Sweeney et al., 2010a). On the other hand, a reduction in the percentage of milk offered instead of drastic removal has proved to be a favorable alternative, in which the reduction of milk is compensated by increasing starter intake. Hence, poor weight gain is avoided by receiving adequate energy to maintain normal growth, in addition to the reduction in the behavioral response. (Khan et al., 2007; Sweeney et al., 2010b; Eckert et al., 2015; Meale et al., 2015; Zobel et al., 2020). Young ruminants early in life are often exposed to significant environmental and immunological stressors, which increase herd mortality and generate economic losses. Thus, feeding strategies that include liquid and solid with high quality nutrients may improve animal welfare, increase productivity, and ensure successful rearing for herd replacement, necessary to reduce mortality rates (Naud and Vasseur, 2021; Todd et al., 2019).

1.6 Weaning

During weaning period, the dam is separated from their progeny in order to reduced their dependence on mother's milk (Mulley et al., 1990). This procedure helps to optimize herd productivity and enhance the quality of newborn ruminant by shifting to a forage, grain-based, or a combination diet independent of the milk doe; which allows the dam to recover her energy reserves on time to breed the following season (Redden and Thorne, 2020). The time of weaning can vary based on factors such as age or weight of the animal; in general most producers target weaning weight at 15 kg at about 45 days of age for breeds such as Alpine or Saanen (Hart and Delaney, 2016; Lu and Potchoiba, 1988; Magistrelli et al., 2013). The separation from the mother and the change of diet is considered a difficult period regardless of species in terms of health and behaviour (Gharib et al., 2018; Habich et al., 2020; Melo and Ungerfeld, 2016, 2016; Moeser et al., 2017). Goat kids experience stress, growth retardation, and weight loss during weaning

(Owens et al., 1993). Step-down milk protocols have been proposed to make a gradual transition to solid feed. When compared to abrupt milk withdrawal, which causes growth depression. It has been demonstrated that step-down has favorable effects on ME intake and BW gain in post-weaning animals. (Steele et al., 2017).

In addition to ruminal development, the weaning period is a time when animals are susceptible to infectious diseases. In fact, in North America, according to NAHMS (2007), one of the causes of mortality and morbidity in young ruminants during the first weeks of life is infectious or parasitic disease (Dwyer et al., 2016) common in housed systems with poor cleaning practices in beds. In goat kids, around weaning, pneumonia is one of the most commonly reported causes of mortality (Naud and Vasseur, 2021; Blanchong et al., 2018). Diet shifts depress immunity as well, and this occurs because the consumption of solid diet contribute to decrease ruminal and intestinal pH, therefore, increases permeability (Liu et al. 2019).This allows the entry of bacterial antigens through ruminal and intestinal epithelium promoting inflammatory reaction (Aschenbach et al., 2019). Weaning also has effects on the lower gut. A study conducted by Steele et al., 2017 with abruptly versus gradually weaned calves reported that early weaning animals had high fecal starch, elevated fecal Lipopolysaccharides (LPS), and systemic inflammation (leaky gut). These characteristics indicate a low dietary efficiency, drawing additional energy to mount an inflammatory response at the expense of growth. In the lower gut antigens leak (Araujo et al., 2015), possibly because cell tight-junctions and receptors detect molecular-associated microbial patterns that are affected by the time of weaning. Studies conducted by (Penner et al., 2009; Wood et al., 2015) showed that late weaning reduces antigen permeability and better modulation in trans-cellular transport. Hence, weaning age can impact the success of the transition to entirely solid feed. Weaning age has been the subject of many studies in order to find the moment in which the rumen and the lower gut are fully functional and do not compromise performance of animals (Blanco et al., 2009; Eckert et al., 2015; Hohenshell et al., 2000; Meale et al., 2017; Palma and Galina, 1995; Steele et al., 2017). Goats naturally wean at more than 4 months of age (Lu and Potchoiba, 1990). In dairy productions, weaning time is often done before natural weaning would occur. The farmer determines the day of weaning for economic reasons. In the case of wild goats, interest in other types of feed begins around 5 to 7

weeks, and milk dependence decreases (Bungo et al., 1998). The traditional weaning time in goats, sheep, and lambs, in some dairy operations ranges between 1 through 30 days of age, this is considered as early weaning. Other producers prefer 60 days old, popular and common weaning time in North America (Haenlein, 2001). In pasture-based systems, where bountiful forages are available, the age of weaning ranges between 90 to 120 days of age. Literature available in goat production suggests at least 6 weeks in milk with a gradual reduction during weaning while kid is adapted to consuming starter feed (Hart and Delaney, 2016). Solid feed intake should reach around 250 g /day and body weight of around 10 kg before removing milk completely (Hart and Delaney, 2016). Body weight at weaning is a crucial criterion, since it affects an increase in weight later in life. Blanco et al., (2009) have found a correlation between weight and weaning age, with adulthood performance. For this reason some producers use weight gains as a reference to wean kids (Raya, 2007; Lu and Potchoiba, 1988).

In the available literature on goat kids, authors such as (Palma and Galina, 1995; Panzuti et al., 2018) have shown that early weaning decreases ADG, and delays growth even later in life at 12 weeks of age. Early weaning (6 weeks of age) is an early moment in which the animals have not included enough concentrate in their diet. When the amount of energy is reduced, the gastrointestinal development is affected, and the proper nutrients absorption of the gut is depressed. (Eckert et al., 2015; Greenwood, et al., 1997). On the other hand, there has been growing evidence that animals weaned in weeks considered as late (i.e between 10 to 12 weeks of age) with a high feeding plan (Meale et al., 2015) show greater growth (Hart and Delaney, 2016), high intake of ME (Eckert et al., 2015), high rumen fermentation i.e VFAs production, rumen development (Quigley et al., 1991; McCoard et al., 2019), and fewer signs of stress.

Delay weaning age with withdraw milk protocols allows for a less traumatic transition to solid feeds. However, this is not always economically feasible for farmers. Interaction between weaning age and weaning method has not been well understood. This interaction is required to develop strategies that avoid growth drop during weaning and ensure a fully developed digestive system.

1.7 Metabolic Rumen Development

The increases in rumen mass could be stimulated by feed, which promotes papillary development and is correlated to the production of VFAs (Baldwin and Connor, 2017). The VFAs come mainly from plant fermentation, hemicellulose, cellulose, and lignin. In addition to starches present in the diet, acetic, propionic, and butyric are the predominant VFAs and they represent 63% and 80% of the metabolizable energy (ME) use in the adult ruminant (Aschenbach et al., 2011; Bergman, 1990).

VFAs are weak organic acids present in two different mechanisms of absorption. VFAs, are in an associated form (VFA^-), which means they are associated with a proton (H^+), or they are in a undissociated form (HVFA) (Besten et al., 2013). Rumen membrane between rumen wall to the blood vessels has lipophilic characteristic permeable to undissociated VFAs only. Then the absorption occurs by passive diffusion. The presence of H^+ molecules in the rumen membrane furnishes passive diffusion. Undissociated form (HVFA) will dissociate in the cytosol releasing VFA- and H+. The proton (H+) released must be removed from the cell or neutralized in order to maintain intracellular pH and rumen tissue integrity (Aschenbach et al., 2011). Absorption of VFAs is influenced by different factors, such as intraruminal equilibration of VFAs between rumen and rumen membrane or papillae permeability (Storm et al., 2012). Furthermore, in the ruminal epithelium, proteins that physically translocate the VFAs across the rumen wall include sodium-hydrogen exchangers (NHE), Sodium/bicarbonate cotransporter (NBC), monocarboxylate transporters (MCT) and anion exchanger isoform (AE) (Graham et al., 2007) (Figure 3). These proteins are found in different parts of the forestomach but are present in a greater extent in the rumen. MCT protein isoforms are identified as MCT1, MCT2 and MCT4, these isoforms transport protons such as basolateral H+ and lactate anion. Also, the monocarboxylates, such as propionate, acetate and butyrate are transported through the rumen membrane (Aluwong et al., 2010). In the basolateral membrane of gastrointestinal epithelial cells, isoforms contribute to intracellular pH-homeostasis regulation in the ruminal epithelium and mediate bicarbonate (HCO^-) export from the gastric mucosa after intracellular alkalinization by a decrease of pH (Bilk et al., 2005). Sodium-hydrogen exchangers catalyzes the exchange of extracellular Na^+ for intracellular H^+. This helps to remove the majority of intracellular proton to maintain intracellular pH

homeostasis (Yang et al., 2012; Yan et al., 2018). Monocarboxylate transporters are responsible for the reabsorption of bicarbonate from cell to the blood, the electrogenic Na^+ / $HCO3^-$ cotransporter carries a net negative charge, provides an uptake of HCO_3^- (Kirat, 2016), and afford, a neutral intracellular pH in ruminal epithelium in acidic environments (Huhn et al., 2003). All these transport and balance mechanisms are crucial to maintain the intraruminal and epithelial health in the animal. The more abundant these proteins are, the faster VFAs will be transported across the rumen wall (Laarman et al., 2016). Protein-mediated VFAs transports are correlated with the abundance of intercellular pH regulator, when the pH of the ruminal liquid is close to neutrality (i.e. 7.0) all the VFAs are absorbed in the same rate, when the pH is low around (i.e. 6.0) the absorption decreases (Dijkstra, 1994).

Rumen concentration of VFAs also plays a role in epithelial cell proliferation via regulation of the cell cycle progression. As evidenced by Sun et al., (2018), VFAs increased gene expression of cyclin A, cyclin D1 and CDK2. Similarly, Gorka et al. (2018) found that butyrate increases papillae development and the epithelium thickness. To accomplish this, butyrate has to enter into the cell to be used as a substrate for its growth (Liu et al., 2019). In addition to nutrient absorption, the rumen epithelium maintains the barrier against possible harmful substances from ruminal bacteria. According to Zhan et al. (2019), in rumen epithelial cells, VFAs regulates innate immune responses. Through microbial metabolites, such as Toll-like receptors, nucleotide oligomerization receptors (NLRs), C-type lectin receptors and RIG-1-like; VFAs activate GPR41 in epithelial cells. This regulatory effect mobilizes polymorphonuclear leukocytes from lamina propria as a defense line for free LPS released from gram-negative bacteria in the rumen.

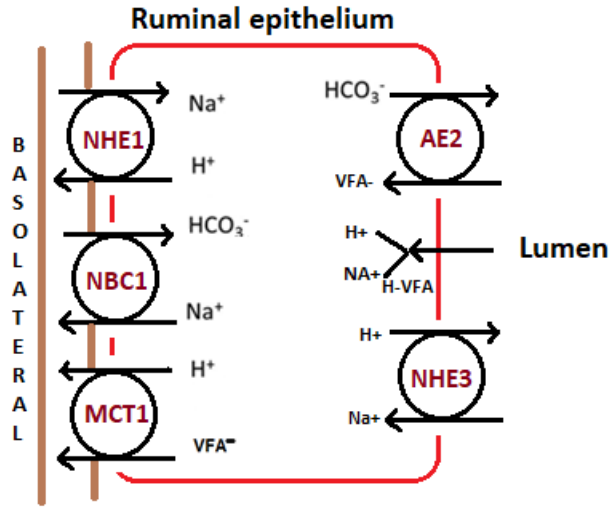

Figure 3. Transport mechanisms of (VFAs) in the rumen epithelium, an adaptation of Laarman et al., (2016)

While ruminants ages, hay and concentrate intakes increase, and linearly, concentration of total VFAs (McCurdy et al., 2019). The type of solid feed, the same as rumen bacteria, affects the ratio of the different VFAs (Dieho et al., 2016). When diet changes, the ruminant in the adult phase needs to adapt its epithelial tissue for the transport of VFAs, and maintenance of ruminal epithelial homeostasis. The drastic increase in concentrations of VFAs without cellular metabolic capacity will affect the integrity of the ruminal tissue (Bannink et al., 2008). The ability of the rumen to absorb VFAs is associated with the resistance and prevention of common diseases in ruminant operation such as subacute ruminal acidosis by highly fermentable diets (Penner et al., 2009).

1.8 Rumen microbial colonization

The rumen is constituted with a diverse microbial community highly specific; that is related to metabolic functions, which are essential for the development, health, and nutrition of the ruminant. Rumen colonization in the newborn starts during the first hours of life, through the dam's birth canal, saliva, partners, feed, and housing (Barden et al., 2020; Jöbstl et al., 2019). The microbial composition of the young ruminant is age dependent. As reported by Lei et al., 2018, goat kids at 3 days of birth exhibited low bacterial diversity relative to adult animals with a predominance of phyla such as Bacteroidetes (45.46 %), and Proteobacteria (27.29 %). This is in

accordance with Jami et al., (2013) who observed a shift between taxa with aerobic function such as *Actinobacillus*, *Gemella, Streptococcus, Mannheimia* to taxa with predominant anaerobic functions, such as *Bacteroides*, *Fusobacterium* and *Coprococcus* during the first week of life. The presence of these taxa contributes to the elimination of oxygen that will create a beneficial environment for the growth of taxa from phyla such as Firmicutes and Bacteroidetes that mostly dominate the gut microbial community in anaerobic conditions (Shin et al., 2015). Feeding regimes in ruminants are represented by microbiota characteristics that are correlated with phenotypes such as major nutrient intake and VFAs concentration. The low abundance of bacteria in early life is possibly due to the absence of solid feed (hay and concentrate), which increases microbial diversity (Van Soest, 1994). As the ruminant ages, milk nutrients, such lactose are less available due to the increase in feeds with complex carbohydrates (starch and cellulose). Hence, the gut microbiota starts to be composed by exclusively the genus *Prevotella* that has been reported prominent in starter feed-fed ruminants (Wang et al., 2016). Similarly, previous studies by Dias et al., (2018) also observed a decrease in genera including *Parabacteroides* and reported the presence of bacterial genera in calves at 28-day-old such as *Bulleidia,* with saccharolytic capacity, and *Succiniclasticum* a succinic acid consumer in transition feed period. The variability and fluctuation of the ruminal microbiota thus lays a solid foundation for the transition from the pre-ruminant to the ruminant state. Many experiments have been conducted in the early stages of life to stimulate ruminal growth by inoculating with adult ruminants bacteria. (Soto et al., 2013; Belanche et al., 2020; Yu et al., 2020; Palma-Hidalgo et al., 2021). Since it has been shown that accelerating microbial colonization of the rumen during the pre-weaning period leads to higher protozoal numbers, a greater bacterial diversity, and minimized stress during the weaning period. Rumen colonization has been proposed to occur in 3 stages: 1) development of microbes during the first 14 days, 2) substantial transition of microbiota colonization between 14 and 28 days, and finally 3) stabilization of the microbial population between 28 and 56 days (Zhang et al., 2019). At two months of age, when solid feed has increased significantly, bacteria of the genus *Prevotella, Lachnospiraceae, Ruminococcus* and *Butyrivibrio* belonging to the phylum Bacteroidetes are present, also but to a lesser percentage, bacteria such as *Eubacterium, Oscillospira* from phylum Firmicutes and Succinivibrio from phylum Proteobacteria, have been reported to stabilize to two

years of age (Jami et al., 2013) and they will be representative together with genera not reported in this manuscript in the population of rumen bacteria community. They will play an important role in carbohydrate and nitrogen metabolism, and the production of VFAs. Close to 3–4% of the rumen microbiome belongs to archaea populations, of which methanogens are the main members which are less sensitive to the changes in age and diet, (Nagaraja, 2016). The presence of methanogens in the rumen in early age is possibly due to interactions with the environment, but its real establishment occurs when the availability of hydrogen (H^+) is sufficient (Wang et al., 2016a).

Rumen fungi represent approximately 10–20% of rumen microbiota, they are vertically transmitted from the mother to the offspring, but similarly to archaea, they are established when the rumen is completely anaerobic and the presence of solid feed is significant (Dias et al., 2017). As an important component of ruminal microbiota, fungi have amylolytic and proteolytic activities and they are the highly active organisms in fiber digestion mainly due to their efficient and diverse types of enzymes capable of degradated structural polymers of plants (Gordon and Phillips, 1998).

Ruminal protozoa are classified into flagellates and ciliates. Ciliates represent the majority of protozoa in the rumen (Nagaraja, 2016) and their colonization depends on direct contact with adult animals. Nevertheless, Minato et al., (1992) observed that in Holstein calves, the colonization of ciliated protozoa occurs between 8 and 10 weeks of age, which means that their presence is strictly linked to the sufficient establishment of other microorganisms such as bacteria and fungi to survive (Fonty et al., 1988). These microorganisms contribute to carbohydrate breakdown and produce H^+ and enzymes such as pectinesterases, cathepsins and some glycosyl hydrolases (Williams et al., 2020). Furthermore, the production of H^+ will be later used by methanogens for growth and finally the production of CH_4, being responsible for 9 –25% of methanogenesis in rumen fluid (Schonhusen et al., 2003). Among other activities in the rumen, protozoa predate bacteria and use them as a source of protein, decreasing the passage to the duodenum and allowing a negative impact on energy efficiency of the rumen ecosystem (Newbold et al., 2015). Therefore, it is possible to affirm that the colonization of the rumen is sequential due to the changes in the animal's diet, which are generated while the animal ages in order to make the rumen an efficient fermenting chamber.

Chapter 2 – Scientific Manuscript

Late weaning improves growth performance and rumen development in Alpine goats.

Formatted for submission to Journal of Dairy Science.

2.1 ABSTRACT

The present study aimed to determine the effects of weaning age on growth performance, rumen development and microbiota composition in Alpine goat kids. Seventy-two kids were randomly assigned in pairs male and female to one of three treatments. 1) early weaning, at 6 wk of age (EW), 2) medium weaning, at 8 wk of age (MW) and 3) late weaning, at 10 wk of age (LW). Milk replacer (MR) was offered *ad libitum* until the step-down wk, when milk was reduced by 12.5% per day for seven days. Two wk after birth, starter ration, hay, and water were offered *ad libitum* until 12 wk of age where kids were slaughtered. Feed intake was recorded daily, and body weight (BW) was recorded weekly. To evaluate rumen development, blood samples were taken during the whole trial and analyzed for blood β-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA). Ruminal measurements such as papillae, VFAs and microbiota were taken at wk 12 with only thirty-six males. Rumen contents were obtained for bacteria composition analysis using the V4 region of the 16S rRNA gene and qPCR was used to quantify bacteria, protozoa and fungi. Rumen papillae were obtained for histological analysis in 4 rumen areas: rumen atrium (RA), ventral sac (VS), caudodorsal blind sac (DS), and caudoventral blind sac (VBS). Kids weaned at 10 wk, compared with 8 wk and 6wk had higher average daily gain for the wk postweaning (0.35 vs. 0.24 vs. 0.24 kg / d) and were heavier at wk 12 (27.44 vs. 25.45 vs. 24.07 kg, $P < .05$). In LW animals, BW was not affected during post weaning period; possibly due to high metabolizable energy (ME) intake caused by the weaning strategy contrary to what was observed in EW and MW animals. Blood levels of BHB increased at weaning time for all treatments but were higher in EW compared to MW (+21%) and LW (+41%) kids ($P < .05$). Blood levels of NEFAs in EW kids spiked at weaning

and were higher than MW (+ 40%) and LW (+101%) in kids (*P*< .05), suggesting a more pronounced adipose tissue mobilization in early weaned kids at weaning. Microbiota population in postweaning showed that while weaning was delayed, abundance of total bacteria seemed to increase (*P*< .05) compared with EW animals, whereas protozoa and fungi decreased for LW. Weaning influences the ruminal microbiota during postweaning. Dual-energy X-ray absorptiometry (DEXA) measurements of carcasses were used as an indication of fat composition recovery in post-weaning. Fat tissue (%) in LW was (9.4%), and EW was (7.2%) higher compared to MW (*P*< .05). The total papilla surface area was greater in LW, compared to MW (+49%) and EW (+22%) kids (*P* <.05). Overall weaning kids at 10 wk of age limited the negative impact of earlier weaning on growth and rumen development in the Alpine breed.

Key words: Goat kids, weaning, rumen development, growth performance.

2.2 INTRODUCTION

The success of any replacement program in dairy goat production depends on raising and developing animals that reach an optimal size and weight to initiate puberty, breed easily in appropriate age and at the lowest possible cost (Lu and Potchoiba, 1988). The digestive system of ruminants undergoes changes between birth and the moment in which the rumen becomes functional. Nutrient absorption changes are observed during the transition from milk to solid feed. Such dietary change alters the pattern of nutrients delivered to the intestine and the liver, and thus to the peripheral tissues of the animal (Baldwin et al., 2004).

The dramatic shift at weaning, when milk replacer is completely removed, triggers rumen microbiota changes and fermentation process. Resulting from ruminal microbial fermentation, volatile fatty acids (VFAs) are produced and then stimulate ruminal papillae development, muscular development, and expansion of ruminal volume, providing niche environments for microbiota able to process highly fibrous feedstuff (Weary and Keyserlingk, 2011; Meale et al., 2017). Butyric acid, which is transformed into β-hydroxybutyrate (BHB) in the rumen epithelium, is one of the major end products of bacterial carbohydrate fermentation and is recognized as a molecule that regulates differentiation and proliferation of rumen tissue (Baldwin and Connor, 2017), which is in turn necessary for optimal absorption of VFAs.

Weaning stress can reduce feed intake and consequently negatively impact growth. Importantly, the age at which the animal is weaned can modulate these effects, possibly due to better ruminal and intestinal development (Eckert et al., 2015). However, to our knowledge, there are no published studies that established optimal weaning age based on growth performance, histomorphology, and microbial shifts in the rumen of goat kids. For this reason, the objectives of this study were to: 1) Evaluate the effect of weaning at 6 (EW), 8 (MW) and 10 (LW) wk of age on growth performance and rumen development, 2) Characterize the differences in rumen microbiota occurring as a result of different weaning ages. We hypothesized that late weaning age improves growth performance, rumen development and ruminal microbiota in kids fed milk replacer.

2.3 MATERIALS AND METHODS

2.3.1 Experimental design and animal housing

Fifty Alpine goats from the Centre de recherche en sciences animales de Deschambault (QC, Canada) were raised to obtain 72 kids (36 females and 36 males). The kids were removed from their dams at birth, were blocked by BW and birth date and penned according to a complete block design including 12 blocks of 3 pens each (with 2 kids per pen, one female and one male). Within each block, pens were randomly assigned to one of three treatments 1) Early weaning at 6 wk of age (EW), 2) Medium weaning at 8 wk of age (MW) and 3) Late weaning at 10 wk of age (LW). All procedures involving goat kids were approved by the local institutional animal care committee in accordance with the guidelines of the Canadian Council on Animal Care (#CL362, 2009).

2.3.2 Colostrum, Milk Starter, Hay and Concentrate

During the first 3 days, kids received a colostrum replacer (LambGro / KidGro colostrum, Grober Nutrition, ON) immediately after birth, six hours and twelve hours later. A feeding system with two gate-mounted natural flow teats (Milk Bar Lamb & Kid Teat, Milk Bar, New Zealand) per pen was used to prevent competition and provide milk replacer *ad libitum*. Fresh milk replacer was offered twice daily (9h00 and 16h00) in a 9-L bucket placed outside the pen. The artificial teat

feeding setup was similar to that previously described (Deelen et al., 2016; Horvath and Miller-Cushon, 2017). Once daily in the morning, after feeding, all buckets, lines, and teats were replaced for clean ones. The milk replacer powder used for the study was Caprilait kid milk replacer (Grober Nutrition, Cambridge, ON) (24.26% CP, 19.76% fat; ME= 4.7 Mcal/kg), reconstituted at 150 g/L in water according to the manufacturer instructions and supplemented with 6.8 g/L acetic acid to preserve the bacteriological quality until the next change. Milk replacer was prepared at each feeding in sufficient volume to feed all kids. From birth to d 85, pelleted kid starter (Goliath VO-21 Deccox, La Coop Novago, Joliette, QC) (86.24 DM; 19.80 CP; ADF 9.28; NDF 24.40; fat 3.89; ME= 2.35 Mcal/kg), fresh chopped hay (83.16 DM; 10.95 CP; ADF 33.10; NDF 55.25; fat 2.01; ME=1.26 Mcal/kg) and fresh water were provided *ad libitum.*

2.3.3 Intakes and Sampling

Milk replacer was recorded and calculated twice daily to maintain *ad libitum* offer to the kids until their respective time of step-down weaning for every treatment. Intakes of starter and hay were recorded daily. Kids were weighed at the same time of day (1300 h) weekly starting at birth until d 86.

Blood was collected from kids in the same day and at the same time as the weight measures starting at 24h of birth until the day of the slaughter (d 86), weekly, by jugular venipuncture using a 21-gauge, 1.5-inch (0.8 X 38 mm) hypodermic needle (BD Vacutainer Precision Glide, Becton Dickinson and CO, Plymouth, UK), into a sterile, plastic Vacutainer tube with anticoagulant (BD Vacutainer K2 EDTA (K2E), Becton Dickinson and CO). Blood was centrifuged at 1000 *g* for 10 min at 4°C, plasma was separated and stored at −80°C until the analysis for BHB at the Diagnostic Center of the Faculté de médecine vétérinaire, Université de Montréal.

2.3.4 Euthanasia and rumen wall sampling

Thirty-six male Alpine kids were slaughtered via captive bolt stunning and exsanguinated at 12 wk postweaning; blood samples and weights were taken before the slaughter. An incision was made along the ventral midline of the kid to expose the gastrointestinal tract. Once exposed, the tract was ligated in the cardiac sphincter and again in the pyloric sphincter and removed from the

abdominal cavity according to the method by Lesmeister et al., (2004). The forestomach was dissected together and weighed full, the reticulum, rumen, omasum an abomasum were weighted separately after being rinsed with distilled cold water. The rumen was opened with an incision of approximately 15 cm starting from the dorsal side of the esophagus crossing the dorsal sac. The reticulo-rumen was laid flat, creating a roughly symmetrical right and left sides separated by the portion of the rumen maintained intact, then, four anatomical areas were selected for papillae development analysis: (1) rumen atrium (RA), (2) ventral sac (VS), (3) caudodorsal blind sac (DS), and (4) caudoventral blind sac (VBS).

2.3.5 Histological Evaluation and Histomorphometry of Rumen Wall Samples

Patches of 3X3 cm^2 were cut from each four areas and cleaned carefully with distilled water, immediately immersed in plastic pots (120 mL) filled with phosphate-buffered 4% formaldehyde solution (pH 7.4) for 24h; then the tissues were placed 48h after in PBS. The samples were used to determine papillae length, width, muscle layers and papillae density (number / cm^2), using the "forbidden lines method" (Gundersen, 1977). Total surface area of papillae per square centimeter mucosa (TSA) was determined as length × width × 2 × papillae density (Ragionieri et al., 2016). The tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 5 µm, stained with hematoxylin-eosin-phloxine-saffron (HEPS) and scanned using an Aperio ScanScope slide scanner (Aperio Technologies, Leica Biosystems, Concord, ON). Complementary software was used for measurements (ImageScope Version 11.2.0.780, Aperio Technologies, Leica Biosystems). Starting at the top left of the slide, six papilla per rumen sections were evaluated.

2.3.6 Animals and DXA scanning

After euthanasia, Dual energy X-ray absorptiometry (DEXA) was used to determine body composition in 36 male Alpine kids using an X-ray bone densitometer system (Hologic Discovery-W, Hologic, Waltham, MA, USA). The whole-body scan mode was used on all animals.

Measurements made by DEXA included total tissue mass (TTM), lean tissue mass (LTM) and fat tissue mass (FTM).

2.3.7 VFAs analysis

Rumen liquid was taken and the whole rumen content was squeezed by cheese-cloth filtrate after slaughter and immediately pH was measured. Rumen liquid samples of 10 mL was directly added in a vial containing 0.2 mL H_2SO_4 (50%, v/v) and stored at −20°C for subsequent VFAs analysis. The VFAs ruminal profile was determined by a gas chromatograph (Agilent 6890N; Agilent Technologies Canada, Mississauga, ON) equipped with a HP-INNOWax capillary column (30 m, 0.32 mm, 0.25 µm) and a flame ionization detector (Agilent Technologies Canada), as described by Baumann et al., (2016). All ruminal fluid samples were assayed in duplicates.

2.3.8 DNA extraction and amplification

Rumen contents, liquid and solid, were collected immediately after slaughter of each kid at d 85. All the contents were mixed well and squeezed through a cheese cloth to separate the rumen content in liquid and solid fractions. Solid and liquid fractions were immediately transported on dry ice and then stored at −80°C. Solid samples were lyophilized (Consol 25LL, VirTis, Gardiner, NY) and mixed in a coffee grinder (Oster, Brampton, ON) for 1 min. Liquid samples were thawed on ice, vortexed and a subsample of 2 mL was taken in a sterile microcentrifuge tube and centrifuged at 17 000 g for 15 minutes. The supernatant was discarded, and 0.5 g of the resulting pellet was used for DNA extraction. DNA was isolated using a commercially available kit (QIAamp Fast DNA Stool Mini Kit, Qiagen, Toronto, ON) with modifications as described by Rico et al. (2015). DNA quality was determined by electrophoresis on a 1.5% agarose gel. DNA was quantified using Qubit® 2.0 Fluorometer and Qubit dsDNA HS assay kit (Life Technologies, Carlsbad, CA), according to the manufacturer's instruction.

2.3.9 16S rRNA sequencing

Sequencing libraries of the 16S rRNA gene were prepared according to the Illumina 16S Metagenomic Sequencing Library Preparation Guide. Briefly, the 16S V3-V4 hypervariable region was amplified using primers (5'-CCTACGGGNGGCWGCAG-3') and (5'-

GACTACHVGGGTATCTAATCC-3') containing Illumina overhang adapter sequences (5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3') and (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3') respectively with KAPA HiFi HotStart ReadyMix (Roche, Wilmington, MA). 2 µl of PCR products were electrophoresed on 1% agarose gel to verify amplification/product size (~513bp) and purified with AMPure XP beads (Beckman Coulter, Indianapolis, IN). Sequencing adapters containing 8-bp indices (N7 and N5 index primers) were added to the 3' and 5' ends by PCR using the Nextera XT Index kit (Illumina, San Diego, CA). Followed by a second purification with Ampure XP beads. The amplicons were quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, Waltham, MA), and equimolar ratios were pooled and combined with 5% equimolar PhiX DNA spike-in (Illumina) for sequencing on a MiSeq sequencing platform instrument, using the 600-cycle V3 MiSeq Reagent kit (Illumina). The sequence files in FASTQ format were processed using Mothur software v.1.44.3, available online: https://mothur.org/wiki/miseq_sop/mothur (Schloss PD et al., 2009). Based on the MiSeq SOP, quality-filtering steps, SILVA v.132 was used as the reference alignment database for the 16S rRNA gene (Pruesse et al., 2007). Sequencing error was reduced using the pre.cluster algorithm and a previous grouping was carried out (dis = 2) to reduce the error. Chimeric sequences were removed using Chimera.Uchime Algorithm (Edgar et al., 2011). Taxa denoted as chloroplast, mitochondria, archaea, eukaryota or unknown were excluded. Sequences were clustered into operational taxonomic units (OTU) based on a 97% similarity cut-off to perform the cluster process. Preliminary visualization, percentage of abundance of the different taxonomic levels, as well as α- and β-diversity analyses were performed using the MicrobiomeAnalyst web platform (Dhariwal et al., 2017). Samples were rarified to the sample with the lowest number of sequences.

2.3.10 Quantitative PCR

The qPCR assays were conducted to evaluate the abundance of total methanogens, eubacteria, protozoa, and fungi. The abundance of these microbial groups was quantified using an AriaMx Real-Time PCR system (Agilent, Santa Clara, CA). Target genes were methanogenic *rrs* (16S ribosomal RNA subunit) for methanogens, eubacterial *rrs* (16S ribosomal RNA subunit) for total eubacteria and *rrs* (18S ribosomal RNA subunit) for total protozoa. Samples of a 100-fold dilution of the DNA extracts from the ruminal liquid fraction were used. Amplicon detection was done

using SYBR Green technology (PerfeCTa SYBR Green FastMix, Quantabio, Beverly, MA). Reactions were set up in duplicate. Each reaction contained 500 nM of each primer and 2 µL of DNA template in a total volume of 20 µL. The primers, annealing temperatures, and PCR efficiencies are listed in Table 7 Supplemental Table S2. Amplification protocols for total methanogens and eubacteria started with a temperature of 95°C for 2 min, followed by 45 cycles of 95°C for 5 s, 60°C for 10 s, and 72°C for 20 s. At the end, melting curve analysis was performed as follows: 95°C for 5 s, 65°C for 1 min, and heating to 97°C with a ramp rate of 0.06°C/ s. Data acquisition occurred 10 times for every °C. For total protozoa quantification, the same protocol was used, but annealing temperature was set to 57°C. Within each run, a standard curve was constructed using 10x serial dilutions (10^1-10^8) of a synthesized double-stranded DNA fragment corresponding to the PCR product (gBlocks Gene Fragments, Integrated DNA Technologies, Coralville, IA) and amplification data were reported as $\log_{10}$ copy numbers per mL of ruminal liquid.

2.3.11 Statistical Analysis

Mixed model regression analysis was conducted using the MIXED procedure of SAS (PROC MIXED, SAS 9.4, SAS Institute Inc., Cary, NC), with time as repeated measures for blood metabolites (NEFA, BHB), body weight, average daily gain, milk intake, kid starter intake and hay intake. Tukey-Kramer adjustment was used to account for multiple comparisons between levels with a heterogeneous compound symmetry (CSH) covariance structure. Results for fixed effects are reported as least squares means ± standard errors according to the following model:

$$Y_{ijk} = \mu + W_i + D_j + WD_{ij} + A_k + \varepsilon_{ijk}$$

where Yijk is dependent variable, µ is overall mean, Wi is fixed effect of treatment (weaning age), Dj is fixed effect of time, WDij is treatment × time interaction effect, Ak is random block (Day and weight of birth) effect, and εijk is experimental error. When not significant, interaction was excluded from the model. All the data were screened for normality using the UNIVARIATE procedure of SAS before analysis. One-way ANOVA was used for analyzes made in wk 12, namely rumen papillae, carcass body composition and VFAs. The α- and β-diversities were compared between groups using primary coordinate analysis (PERMANOVA) and similarity analysis (ANOSIM) within the MicrobiomeAnalyst platform.

2.4 RESULTS

2.4.1 Intake, Growth performance

No significant differences were detected in milk replacer intake during the preweaning period between treatments (Table 1). Starter intake was similar prior to weaning, (Table 2), goat kids had high starter intake right after the milk was removed and a treatment × wk interaction was observed ($P < .05$) across all treatments (Table 1). EW treatment had the greatest increase in starter intake (+485%) while MW increased (+424%) and LW (+391%), in regard to the wk before weaning (Table 2). An increase in hay intake was also observed ($P < .05$) in animals weaned at EW (+1 275%), MW (+288%) and LW (+86%) after milk replacer withdraw. Overall, solid feed intakes were greater for EW (31.9 kg) compared to MW (24.2 kg) and LW (17.3 kg) goat kids ($P < .05$. Table 2) during all the trial. ME intake from milk replacer, starter and hay were calculated from nutritional values and total ME intake was drastically decreased at weaning for all weaning ages (Figure 1). Comparing treatments, total ME intake at weaning was higher when increasing weaning ages (EW, 95 kcal/d; MW, 297 kcal/d; LW, 451 kcal/d) ($P < .05$; see Figure 1).

Body weight was influenced by weaning time (treatment × wk, $P < .05$; Figure 2). Growth in EW kids reached a plateau (Figure 2) at 0.22 kg/d (Table 3) during the step-down wk, marking a decrease (−17%) from the prior wk and continued to a pronounced decline reaching 0.04 kg/d (−83%) during the wk of weaning, and remained the lowest until 12-wk, compared to MW and LW kids ($P < .05$. Figure 2). For MW animals, the growth plateau was much less pronounced during the wk of step-down, with only a −4% reduction from the wk prior milk reduction (0.30 to 0.28 kg/d) and the growth decreased to 0.08 kg/d (−73%) during the wk of weaning (Table 3). Growth in LW animals was not affected during the step-down wk, even increasing (+11%), and the growth decreased similarly to MW to 0.08 kg/d at the wk of weaning (−77%) (Table 3). LW kids increased daily weight gain significantly above EW and MW from wk 8 to wk 12, reaching 0.38 kg/d and overall (0.28 kg/d) ($P < .05$, Table 3). At the end of the trial (12-wk), LW kids had greater body weight (27.44 kg), compared to MW (25.45 kg) and EW kids (24.07 kg).

Plasma BHB concentration showed a treatment × wk interaction ($P < .05$) during the trial (Figure 3A). During the step-down wk, an increase in BHB concentration was observed in EW (+5%), MW

(+8%) and LW (+38%) compared to the wk −1, without any treatment effect. During weaning, BHB concentration in EW had a sharp increase (P< .05) by +21% compared to MW and +41% compared to LW animals. During the postweaning wk, BHB concentrations varied regardless of treatments. During wk 1 postweaning, a reduction was observed in EW (−6%), MW (−2%) and LW (−7%) respectively. At wk 2 in postweaning, the treatments EW (−29%) and LW (−4%) showed reduction in BHB concentrations, contrary to what was observed in MW (+18%). Plasma concentrations of NEFA differed (P< .05) between treatments during the step-down wk. EW had an increase of +9% compared to MW (P < .05) and +58% compared to LW animals (P< .05) (Figure 3B). During weaning, EW had an increase of +40% (P< .05) compared to MW and +100% compared to LW animals (P< .05). In wk 1 post-weaning, EW continued with greater levels of NEFA in plasma compared to MW (+33%, P< .05) and LW (+ 281%, P< .05). In wk 2 postweaning, NEFA concentrations in plasma reached similar levels in preweaning and no significant differences were found between treatments.

2.4.2 Anatomical development, Carcass scanning (DXA) and morphometric characteristics

The forestomach was assessed in male goat kids at twelve wk old. The rumen, reticulum, omasum, and abomasum weights in the different treatment groups are shown in Table 4. Empty digestive system in goat kids in EW was heavier by +13% (862 g) (P< .05) compared with kids in LW (760 g), and by +10% compared to kids in MW (780 g). Likewise, empty rumen weight for EW was +10% (550 g) heavier than MW (500 g) and +14% (489 g) in LW. For reticulum, omasum, and abomasum weights, differences between treatments were not detected. Carcass composition determined by DEXA (Table 4) demonstrated that TTM in LW kids was higher by +15% and +11% (P< .05) compared with EW and MW animals respectively. The LTM was higher for LW animals by +18% and +11% (P< .05) compared with EW, and MW animals respectively. FTM for LW animals was higher by +19% and +21% (P< .05) than EW and MW animals respectively. The DEXA-derived LTM and FTM was strongly related to whole body without rumen, reticulum, omasum, and abomasum.

Fat tissue percentage was higher in LW (13.96%) compared to MW (12.77%) kids (*P*< .05) and was similar to EW kids (13.69%) (Table 4).

2.4.3 Rumen fermentation

Rumen fluid pH and VFAs concentration (i.e., acetate, propionate, and butyrate) are shown in Table 5. Treatment did not affect rumen pH. Acetic acid (C2) production, in MW was greater compared with EW (+15%, *P*< .05) and LW (+17%, *P*< .05) respectively. Also, an increase in propionic acid (C3), concentration was observed in LW compared to EW (+50%, *P*< .05) and MW (+25%, *P*< .05). Total VFAs (tVFAs) did not differ (*P*< .05) between MW kids and LW kids, but a difference was observed in EW kids by −18% (*P*< .05) compared to MW and a trend of −16% (*P* = .06) compared to LW.

2.4.4 Rumen Bacterial composition and qPCR

The number of inputs 16S rRNA sequence reads generated from rumen microbiota were 511.134. Phylogenetic analysis of these sequences identified 8 phyla from the rumen, and were represented, by Firmicutes, Bacteroidetes and Proteobacteria. Relative abundance (% of total 16S rDNA) of the main Phyla are listed in Table 7. Supplemental Table S1. The most abundant phylum in the rumen was Bacteroidetes across the three treatments; EW (50%), MW (49%) and LW (47%). The second most abundant phylum was Firmicutes (EW 38%, MW 40%, LW 42%), and the third phylum Proteobacteria (EW 8%, MW 7%, LW, 7%) without significative differences between treatments (Figure 7. Supplemental Figure S1). In contrast, a trend was observed in phyla less predominant such as Spirochaetes (*P*= .07), EW was greater by +189% compared with MW and by +141% to LW animals. Unclassified bacteria showed differences (*P*< .05) between treatments, MW was greater by +253% and +53%) than LW and EW animals respectively. For Synergistete, decrease was detected in MW and LW animals (−161%, *P*< .05) compared with EW animals. Treatments did not affect the rumen α-diversity (*P*= 0.66). Microbiota richness as indicated by the Chao1 and evenness (Shannon and Simpson) are reported in Table 9 supplemental Table S3 without significative differences between treatments. Rumen β-diversity (*P*≥ 0.258;

PERMANOVA, Figure 8 Supplemental Figure S2) visualized by PCoA analysis (OTU ≥ 97% identity, genus level similarity) using Bray-Curtis index revealed no effect of weaning age.

The qPCR-based test for total eubacteria abundance was greater for animals in LW compared with EW animals ($P < .05$. Table 5). The relative abundance of protozoa significantly decreased for animals ($P < .05$) in LW compared and EW. Weaning time also influenced the abundance of fungi that significantly decreased ($P < .05$) in LW compared to EW. Weaning age had no effect on total methanogens copy numbers.

2.4.5 Rumen development

The 4 anatomical locations (RA, VS, DS, and VBS) are presented in Table 6. In RA, papillae length was greater (MW, +20 %) and (LW, +9 %) compared with EW animals ($P < .05$). Papillae width in RA was (MW, −28%) and (LW, −1 %) lesser than EW ($P < .05$). Papillae length in VS, (MW, −18 %) and (LW, −14 %) lesser than EW animals ($P < .05$). Papillae width in VS was (MW, +15%) and (LW, +58 %) greater than EW. Papillae length in DS, (MW, −9 %) and (LW, −22 %) lesser than EW animals ($P < .05$). Papillae width in DS and papillae length in VBS did not show significant differences between treatments. Papillae width in VBS was lesser (MW, −20%) and greater (LW, +66 %) than EW ($P < .05$). Overall rumen length did not show significant differences between treatments. Overall rumen width showed significant differences ($P < .05$) for animals in the treatment LW. Muscle layer in DS was also significantly greater in EW ($P < .05$) animals without significant differences in the other rumen locations. In the overall muscle layer analysis, differences were not observed between treatments. Papillae density did not show differences except for RA that was greater in + 24% for LW animals compared with MW and EW animals ($P < .05$). Total surface area TSA was lesser (−18%, MW) and greater (+24%, LW) than EW in RA ($P < .05$). In VS was lesser in −7% for MW and greater in +34% for LW than EW ($P < .05$). DS was lesser in −13% for MW and −23% for LW than EW ($P < .05$). VBS was lesser (−32%, MW) and greater (+54%, LW) than EW ($P < .05$). Overall analysis of TSA showed that LW was greater in +22% compared with EW and +48% compared with MW ($P < .05$).

2.5 DISCUSSION

In an attempt to establish techniques that improve production efficiency for the dairy goat industry, this study was conducted to test the hypothesis that late weaning improves the growth performance, rumen development and ruminal microbiota in kids fed with milk replacer. We compared the effects of weaning at different ages, since delaying the age of the milk withdrawal could minimize the negative effects of transition from milk to solid feed on the performance in goat kids (Meale et al., 2015; Campbell, 2018).

In commercial dairy goat operations, weaning age ranges between 5 to 10 wk of age (Naud and Vasseur, 2021; Barkley, 2014). The reference for weaning age is mainly based on the cost of milk replacers (Hart and Delaney, 2016). However, studies investigating the impact of varying weaning age in goat kids are scarce. Interestingly, we observed an advantage in BW and ADG in animals weaned at 10 wk of age. Similarly, a trend for higher daily weight gains was observed by Fehr et al. (1976) in Alpine male kids weaned at 10 wk of age. Therefore, our observations support the view that goat kids may have an advantage in BW when *ad libitum* milk for a longer time and solid feed are offered in the pre-weaning period, which in turn provide higher ME intake during weaning (Figure 4). On the other hand, the lowest ME intake support the idea that early weaned kids were not able to compensate for the reduced milk replacer offer, through increased in starter and hay ME intakes. Although differences in BW at weaning were expected because comparisons were made with animals of different ages 6wk, 8wk,10 wk. Early weaning resulted in reduced growth performance relative to MW and LW at wk 12. Negative effects of EW have been observed in other species as well. For example, bull calves weaned at 8 wk, as an early weaning age, exhibited lower BW at 13 wk of age compared with animals weaned at 12 wk in elevated milk plan (8 L/d) (Meale et al., 2015). High ME intake in late weaned animals allows optimal BW and consequently could initiate their reproductive and productive career sooner. Literature recommends that goats should achieve breeding with 60% of adult weight, at 32 wk of age (Hart and Delaney, 2016). Higher BW and higher milk replacer intake in the preweaning period minimize the use of energy for growth after weaning and, as reported in dairy calves, could positively affect productivity Soberon et al. (2012).

Feed intake data showed that right after weaning day, the MW and LW groups were above the minimum recommended intakes for goat kids (Hart and Delaney, 2016). On the other hand, EW did not reach this recommendation. In contrast to reports by Panzuti et al. (2018), kids weaned at 4 wk of age (9.7 kg BW) had intakes around of 33 g of DM. Physical form of starter feed can affect the palatability, nutrient digestibility and fermentation capacity of animals (Lesmeister and Heinrichs, 2004). This has important implications for the adult ruminant, which will derive up to 80% of its energy intake from ruminal fermentation (Li et al., 2021). Thus, the efficient use of solid feed in the postweaning period will influence growth performance of the animal.

Shift from liquid to solid intake changes the metabolites use for energy, from glucose to BHB (Quigley and Bernard, 1992). Most of VFAs produced by organic matter fermentation is absorbed in rumen epithelium, but 90% of butyrate is transformed to BHB in adult ruminants (Naeem et al., 2012). Circulating BHB in the current study increased after weaning, which could be associated with transition stress or rumen ketogenesis (Lane et al., 2002). Indeed, the conversion of butyrate to BHB in the rumen epithelium is a well-documented characteristic of ruminants with a fully developed and functional rumen (Baldwin et al., 2004; Deelen et al., 2016; Pennington, 1952; Sutton et al., 1963). During the step-down wk, BHB concentrations were consistent with previous reports (Baldwin and Jesse, 1992; Byrne et al., 2017) where BHB concentrations are correlated to increases of starter and hay intakes. Immediately after weaning, animals maintained elevated levels of BHB while NEFAs decreased, regardless of treatment. Our data suggest that elevated post-weaning BHB concentrations may originate from rumen ketogenesis, as previously reported (Lama et al., 2014; Sun et al., 2018), especially animals in LW in which increases in BHB did not change after weaning compared with MW and EW animals. During fasting, ruminants have the ability to perform hepatic ketogenesis in order to use NEFAs as a source of energy by adipose tissue mobilization (Baldwin et al., 2004a). In our study, circulating NEFAs increased as feed intake decreased, which has been previously reported in calves around weaning (Quigley, 1996; Klotz and Heitmann, 2006). A pronounced drop in total feed intake after weaning was observed in all treatments. This weaning stress was associated with increased concentrations of plasma NEFAs. Such elevations were more pronounced in EW and MW. We presume that NEFAs spikes occurred

by a negative energy balance, with mobilization of adipose tissue to produce BHB by liver ketogenesis as a source of energy.

Dual-energy X-ray absorptiometry (DEXA) has been previously used as a tool for the determination of body composition in animal production such as sheep, lambs, pigs and beef calves (Marcoux et al., 2003; Suster et al., 2003; Pearce et al., 2009; Gibson et al., 2020; Calnan et al., 2021). To the best of our knowledge, there is no previous study that has examined long-term effects of weaning on body composition assessed by DEXA. In the current study, we examined body composition at 12 wk of age for all treatments. In LW animals (2 wk after weaning), lean mass and fat mass and % were not affected compared to MW (4 wk after weaning), indicating that LW animals probably did not have to use their energy reserves to compensate for the weaning effect, in accordance with plasma NEFAs levels around weaning. Interestingly, even if NEFAs levels were the highest at weaning, fat % in EW animals were similar LW kids at 12 wk of age (13.7% vs 14.0%), supposing that 6 wk after weaning were enough for EW animals to restore their adipose tissue, whereas 4 wk after weaning were not for MW kids.

The greater empty rumen weight in EW animals is in line with previous studies that demonstrated that large particle size and form of fibrous feed offered during the first wk of life can provide mechanical stimuli to enhance rumen weight (Khan, Weary, and Keyserlingk, 2011). Also, EW animals had more time to consume solid feed in the postweaning period (6 wk) compared with MW (4 wk) and LW (2 wk), which is in accordance with our results of muscular layer thinnest. Growth of rumen papillae is minimal during the non-rumination phase (Baldwin et al., 2004; Baldwin and Connor, 2017) and depends on the intraruminal concentration, timing and duration of VFAs exposure in the rumen epithelium (Lane et al., 2000). In the present study, our sampling schedule limited the possibility to evaluate VFAs changes across the whole trial, and our data collected at wk 12 did not allow us to observe the development from pre-ruminant to ruminant. However, histological analysis showed greater width and larger surface area in LW animals, but no overall effect on length of papillae. In accordance with previous studies in lambs (Carballo et al., 2019) and calves (Khan et al., 2011), this result indicates that higher starter intake and VFAs concentration in the rumen trigger rumen epithelial development in animals weaned late. We speculate that, EW kids did not have the ability to make efficient use of hay and starter feed in

terms of VFAs production and absorption. For this reason, we believe that, in addition to lower total ME intake at weaning, it may have affected the growth performance compared to MW and LW kids, which exhibited an advantage in BW and rumen development.

In line with previous studies (Jami et al., 2013; Meale et al., 2016), the level of stress associated with weaning did not have an effect on alpha and β-diversity of rumen microbial communities in post weaned kids. In the present study, ruminal microbiota was evaluated at 12 wk of age. We were able to observe that, although LW animals had a shorter time (2 wk) before being euthanized relative to the other groups (4 and 6 wk). The whole rumen microbial composition after weaning resulted in an association able to perform all the main fermentative functions. Regardless of the type of rearing, rumen bacteria and methanogens colonization start at birth, as previously reported by Abecia et al. (2014). Likewise, previous publications in goat kids and lambs have reported higher concentrations of fungi when animals are fed with high concentrate-rich diet (Belanche et al., 2020) or during the grazing period in post weaning (Belanche et al., 2019), similarly, to our observations in the EW kids. As suggested by Fonty et al. (1988), protozoa population requires an established ruminal environment, and thus, the change in microbiota between preweaning and post weaning can delay protozoa establishment. In agreement with our results, EW animals had higher concentration of protozoa at 12 wk of age, which may be associated to a longer time-consuming solid feed between weaning and the slaughter day. In accordance with Teather et al., (1984), we observed an inverse relationship between total bacteria and protozoa in LW animals, perhaps due to the competition for nutrients in the rumen and to the predatory activity of protozoa (Foulkes and Leng, 1988), which reduces microbial protein available to the animal (Eugène et al. 2004). This is an advantage for animals in LW in terms of microbial population availability over EW and MW.

2.6 CONCLUSIONS

The results of this study suggest that late weaning in goat kids using a step-down program allows higher nutrient intakes before and during weaning and has a positive impact on growth rates rather than early and medium weaning. Blood metabolites were also indicators of rumen fermentation and energy state at weaning. Therefore, late weaned kids were better prepared to

handle the transition from milk to solid feed. Future research is necessary on feed alternatives that increase fermentation, motility, muscularization and volume of the rumen that trigger rapid solid intake and then ruminal development to optimize growth in the transition from pre-ruminant to ruminant in goat kids.

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Manuscript figures

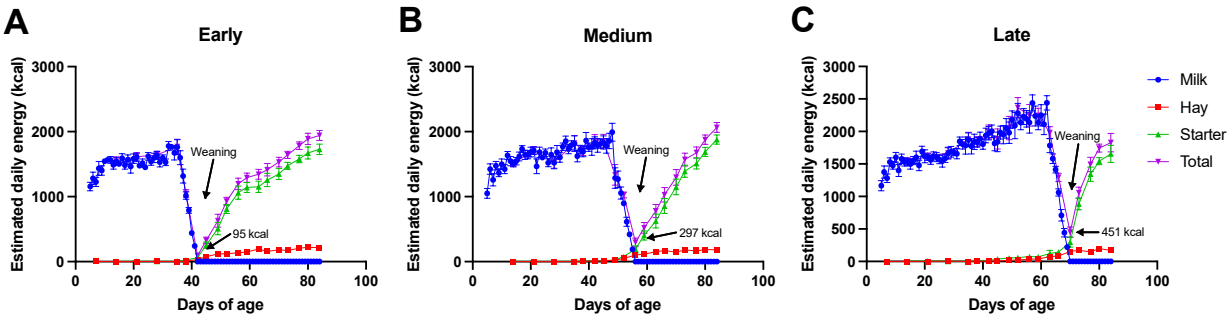

Figure 4. Mean estimated daily ME intake from milk replacer, starter, and hay from d 3 to d 85 of life from kids weaned at 6 (EW) (A), 8 (MW) (B), and 10 LW wk of age (C). SEM is indicated by bars.

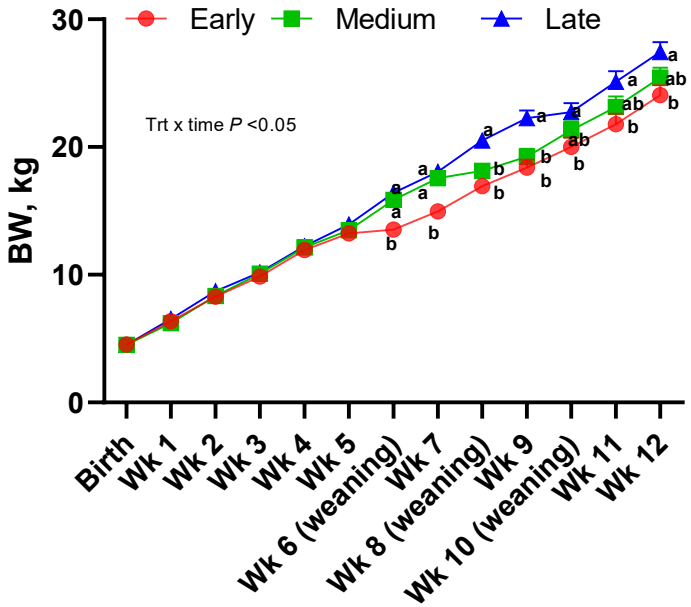

Figure 5. BW of kids weaned at 6 (EW), 8 (MW) and 10 (LW) wk of age from birth to 12 wk. SEM is indicated by bars. [a,b,c] Differing superscript letters indicate weekly least squares means differ between treatments (treatment X time interaction; P< .05).

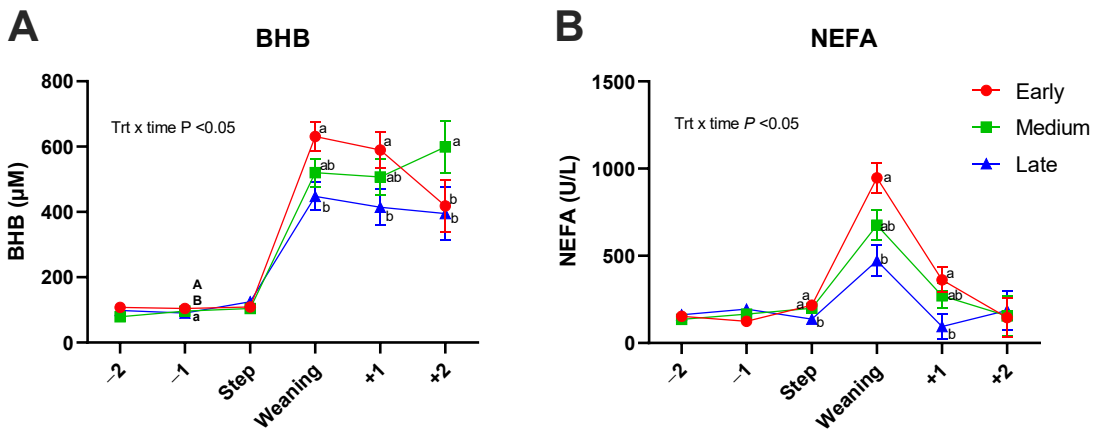

Figure 6. Summary of mean of BHB (A) and NEFA (B) per kids weaned at 6 (EW), 8 (MW) and 10 (LW) wk of age.. SEM is indicated by bars. Step = step down (wk before weaning); Weaning = wk after weaning; −2, −1, +1, +2 = wk relative to weaning. [a,b,c] Differing superscript letters indicate weekly least squares means differ between treatments (treatment X time interaction; P< .05).

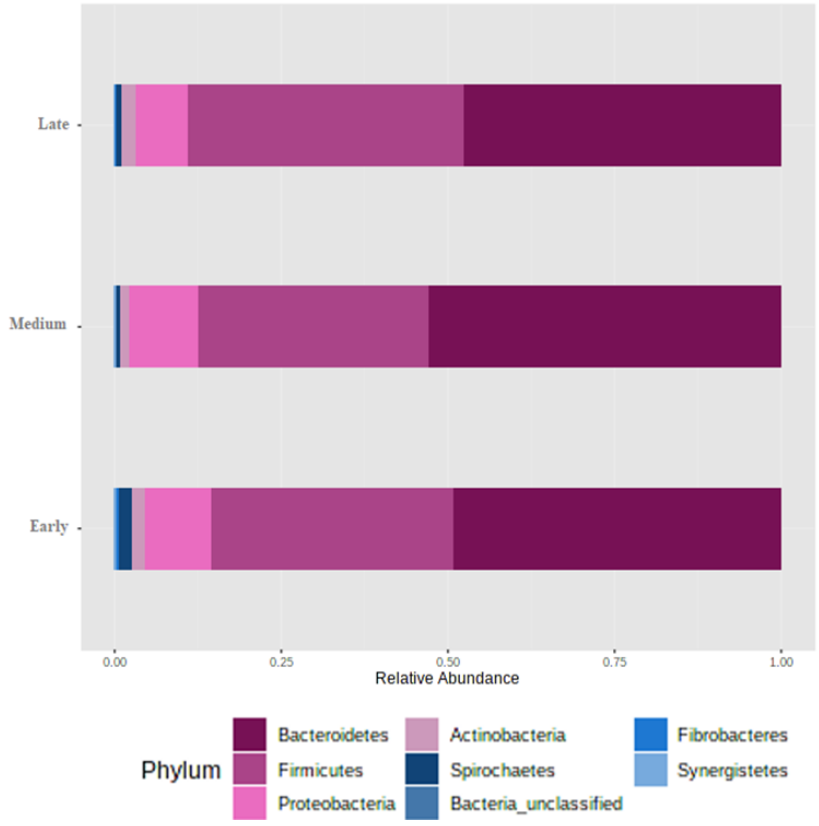

Figure 7 Supplemental Figure S1. Phylum level composition. Bar plots showing average relative abundance of bacterial phyla (%) in the rumen of Alpine male goat kids at 12 wk of age in animals weaned at 6 (EW), 8 (MW) and 10 wk of age (LW).

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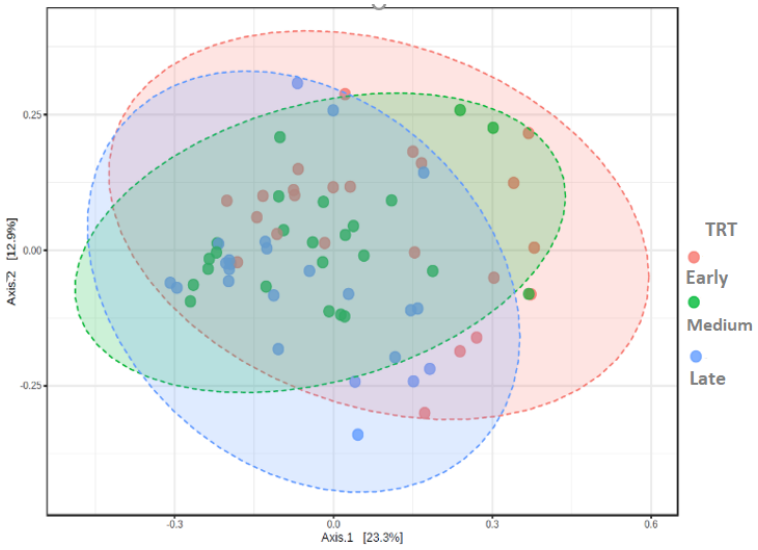

Figure 8 Supplemental Figure S2. Principal coordinate analysis illustrating the bacterial β-diversity dispersion of rumen samples between treatments.. Green dots and area = 8 wk of age (MW). Blue dots and area 10 wk of age (LW) (PERMANOVA: R2 = 0.03; P = 0.25).

Manuscript tables

Table 1. Milk replacer intake1 in goat kids at different wk relative to weaning age (Early, 6 wk; Medium, 8 wk; Late, 10 wk).

Item	Treatment				
	Early	Medium	Late	SEM	*P*-value
Milk replacer, L/d					
Wk 5	2.40	2.53	2.55	0.07	NS
Wk 6 (weaning)	1.13[b]	2.54[ab]	2.70[a]	0.08	< .05
Wk 7	NA	2.55	2.82	0.12	NS
Wk 8 (weaning)	NA	0.92[b]	3.15[a]	0.12	< .05
Wk 9	NA	NA	3.20	0.11	NA
Wk 10 (weaning)	NA	NA	1.10	0.26	NA
Overall, L	77.41[c]	111.39[b]	156.21[a]	4.60	< .05

[a-c]Means within row with a common superscript differ ($P \leq .05$).

[1] Each least square mean is the mean of 12 observations per group of daily intakes of milk replacer, starter, and hay.

Table 2. Starter and hay[1] intakes in goat kids at different wk relative to weaning age (Early, 6 wk; Medium, 8 wk; Late, 10 wk).

Item	Treatment				
	Early	Medium	Late	SEM	*P*-value
Starter Intake, g/d					
Wk 5	3.86	2.94	2.95	0.88	NS
Wk 6 (weaning)	23.45[a]	7.46[b]	9.14[b]	4.10	<.05
Wk 7	192.04[a]	11.01[b]	29.5[b]	24.65	<.05
Wk 8 (weaning)	443.29[a]	73.98[b]	33.86[b]	24.63	<.05
Wk 9	562.68[a]	284.61[b]	56.37[c]	40.61	<.05
Wk 10 (weaning)	639.85[a]	534.99[ab]	95.02[b]	44.35	<.05
Wk 11	752.60	717.23	540.16	29.78	NS
Wk 12	828.08	882.11	777.39	38.32	NS
Overall, kg	3.34[a]	2.52[b]	1.76[c]	0.14	<.05
Hay, g/d					
Wk 5	17.5	6.2	4.9	2.7	NS
Wk 6 (weaning)	88.8[a]	14.7[b]	14.0[c]	6.4	<.05
Wk 7	124.3[a]	67.8[ab]	26.9[b]	12.2	<.05
Wk 8 (weaning)	158.9[a]	127.3[ab]	49.0[b]	14.1	<.05
Wk 9	164.4	163.5	113.8	17.2	<.05
Wk 10 (weaning)	181.1[a]	163.4[ab]	158.2[b]	11.5	<.05
Wk 11	201.6	178.7	179.5	13.7	<.05
Wk 12	208.0	179.4	178.9	18.9	<.05

Overall, kg	6.9[a]	5.31[b]	4.21[b]	0.43	<.05

[a-c]Within a row, means within row with a common superscript differ (*P*≤ .05). [1]Each least squares mean is the mean of 12 observations per group of twice daily intakes of starter, and hay.

Table 3. ADG in goat kids1 at different wk relative to weaning age (Early, 6 wk; Medium, 8 wk; Late, 10 wk).

Item	Treatment				
	Early	Medium	Late	SEM	*P*-value
ADG, kg					
Wk 5	0.22	0.22	0.29	0.04	NS
Wk 6 (weaning)	0.04[b]	0.30[a]	0.29[a]	0.04	<.05
Wk 7	0.24	0.28	0.31	0.03	NS
Wk 8 (weaning)	0.25[a]	0.08[b]	0.31[a]	0.03	<.05
Wk 9	0.24[ab]	0.18[b]	0.35[a]	0.04	<.05
Wk 10 (weaning)	0.21[ab]	0.30[a]	0.08[b]	0.05	<.05
Wk 11	0.29	0.27	0.38	0.04	NS
Wk 12	0.29	0.31	0.31	0.03	NS
Overall	0.24[b]	0.25[b]	0.28[a]	0.01	<.05

[a-c]Means within row with a common superscript differ (*P*≤ .05).

Table 4. Effects weaning age on organs weight and tissue deposition measured by dual energy X-ray absorptiometry (DEXA) of goat kids at 12 wk of age.

Item	Treatment				
	Early	Medium	Late	SEM	*P*-value
Empty digestive system, kg	0.86[a]	0.78[b]	0.76[b]	0.03	<.05
Rumen, kg	0.55[a]	0.50[b]	0.48[b]	0.02	<.05
Reticulum, kg	0.070	0.068	0.063	0.003	NS
Abomasum, kg	0.19	0.16	0.16	0.01	NS
Omasum, kg	0.052	0.048	0.050	0.004	NS
DEXA					
Lean tissue, kg	13.66[a]	14.51[a]	16.14[b]	0.42	<.05
Lean tissue, %	56.84	57.04	57.37	0.53	NS
Fat tissue, kg	3.30[a]	3.25[a]	3.94[c]	0.17	<.05
Fat tissue, %	13.69[ab]	12.76[b]	13.96[a]	0.41	<.05
Total mass, kg	17.45	18.26	20.61	0.58	<.05

[a-c] Means within row with a common superscript differ ($P \leq .05$).

Table 5. Rumen pH, and volatile fatty acids (VFAs) profiles in goat kids at 12 wk of age.

Rumen pH, and volatile fatty acids (VFAs) profiles in goat kids at 12 wk of age

Item	Treatment				
	Early	Medium	Late	SEM	*P*-value
Rumen pH	6.00	5.88	5.74	0.10	NS
Acetic acid, C2, mmol/L	37.34[a]	43.78[b]	37.47[a]	2.07	<.05
Propionic acid, C3, mmol/L	11.37[a]	16.91[b]	22.65[c]	1.87	<.05
Butyric acid, C4, mmol/L	7.18	7.41	6.89	0.70	NS
Valeric acid, C5, mmol/L	1.42	1.76	1.92	0.23	NS
Caproic acid, C6, mmol/L	0.49	0.45	0.34	0.10	NS
Isobutyric acid, isoC4, mmol/L	0.36	0.43	0.33	0.04	NS
Isovalerate, isoC5, mmol/L	0.37	0.42	0.34	0.05	NS
Total VFAs, mmol/L	58.53[a]	71.17[b]	69.95[ab]	4.14	.08
Microbiota, log_{10} copies/mL[1]					
Total eubacteria	9.21[b]	9.38[ab]	9.56[a]	0.09	<.05
Methanogens	8.12	8.19	8.22	0.10	NS
Protozoa	1.75[a]	1.27[ab]	0.98[b]	0.22	<.05
Fungi	3.50[a]	2.99[ab]	2.27[b]	0.39	.009

[1]Abundance of rumen methanogens, total eubacteria, protozoa, and fungi.

[a-c]Means within row with a common superscript differ ($P \leq .05$).

Table 6. Effects weaning age on rumen papillae in goat kids at 12 wk of age.

Item	Treatment				
	Early	Medium	Late	SEM	*P*-value
RA length, mm	3.09[b]	3.71[a]	3.37[b]	0.14	<.05
RA width, mm	0.48[a]	0.34[b]	0.47[a]	0.03	<.05
VS length, mm	2.43[a]	1.99[b]	2.09[b]	0.08	<.05
VS width, mm	0.37[b]	0.43[b]	0.59[a]	0.03	<.05
DS length, mm	2.21	2.01	1.72	0.09	NS
DS width, mm	0.46[b]	0.47[b]	0.56[a]	0.04	<.05
VBS length, mm	1.92	1.95	2.03	0.08	NS
VBS width, mm	0.41[b]	0.33[b]	0.69[a]	0.04	<.05
Overall length, mm	2.41	2.41	2.31	0.06	NS
Overall width, mm	0.43[b]	0.39[b]	0.58[a]	0.02	<.05
Muscle layer, mm					
RA	1.10	1.24	1.13	0.06	NS
VS	1.28	1.12	1.11	0.06	NS
DS	1.57[a]	1.52[a]	1.21[b]	0.08	<.05
VBS	0.93	1.02	1.05	0.06	NS
Overall	1.22	1.22	1.13	0.04	NS
Density, number / cm^2					
RA	83.9[b]	83.8[b]	98.4[a]	5.0	.07
VS	106.9	103.8	96.5	6.8	NS
DS	89.6	85.4	78.8	5.7	NS

VBS	106.1	97.9	98.7	5.8	NS
Overall	96.6	92.7	93.1	3.1	NS
Surface, mm^2 / cm^2 (TSA)[1]					
RA	257.1[ab]	210.1[b]	320.0[a]	23.6	<.05
VS	179.7[b]	167.0[b]	241.0[a]	14.4	<.05
DS	193.2	167.3	148.2	15.4	NS
VBS	182.3[b]	124.4[b]	281.6[a]	23.7	<.05
Overall	203.0[b]	167.2[c]	248.4[a]	10.2	<.05

[1]Total surface area of papillae per square centimeter mucosa (TSA) was determined as length × width × two × papillae density. TSA (mm^2/cm^2), papillae length (mm), papillae base width (mm), muscle layer thickness (mm), papillae density (no./cm^2),) in the ruminal atrium (RA), the ventral sac (VS), the caudodorsal blind sac (DS), and the caudoventral blind sac (VBS). [a-c]Means within row with a common superscript differ (*P*≤ .05).

Table 7. Supplemental Table S1. Relative abundance (% of total 16S rDNA) of the main Phyla.

Item	Treatment				
	Early	Medium	Late	SEM	*P*-value
Actinobacteria	2.42	2.23	2.20	0.49	NS
Bacteroidetes	49.55	48.94	47.02	2.12	NS
Fibrobacteres	0.29	0.08	0.16	0.16	NS
Firmicutes	37.94	40.32	42.85	2.51	NS
Proteobacteria	8.04	7.45	7.03	1.95	NS
Spirochaetes	1.40	0.48	0.58	0.30	.06
Bacteria_Unclassified	0.30[b]	0.48[a]	0.13[b]	0.08	<.05

Synergistetes	0.06[a]	0.02[b]	0.02[b]	0.01	.07

[a-c]Means within row with a common superscript differ ($P \leq .05$).

Table 8. Supplemental Table S2. List of primers for real-time quantitative PCR.

Target	Forward	Reverse	Annealing T (°C)	PCR efficiencies DNA|cDNA	Author
Total protozoa	GCTTTCGWTGGTAGTGTATT	CTTGCCCTCYAATCGTWCT	55	82.8%	Sylvester et al. 2005
Anaerobic fungi	GAGGAAGTAAAAGTCGTAACAAGGTTTC	CAAATTCACAAAGGGTAGGATGATT	60	102.2%	Denman and McSweeney 2006
16S rRNA total eubacteria Bac338F/Bac518 R	ACTCCTACGGGAGGCAGCAG	ATTACCGCGGCTGCTGG	60	93.5%	Ovreås et al., 1997
16S rRNA total methanogens Met630F/Met803 R	GGATTAGATACCCSGGTAGT	GTTGARTCCAATTAAACCGCA	60	83.9%	Hook et al., 2009

[1]Selected forward (F) and reverse (R) primers validated for genes encoding 16S ribosomal RNA (bacterial species and fungi), and for the 18S ribosomal RNA gene (protozoa).

[2]Annealing temperature.

Table 9. Supplemental Table S3. diversity indexes in the Bacteria community in the rumen of alpine goat kids weaned at different ages.

Item	Treatment				
	Early	Medium	Late	SEM	*P*-value
Chao1	59.24	56.47	57.11	2.27	NS
Shannon	2.44	2.38	2.36	0.04	NS
Simpson	0.83	0.82	0.82	0.01	NS

[a-c]Means within row with a common superscript differ ($P \leq .05$).

Chapter 3- General Discussion

With this thesis, we present a set of observations of multiple aspects related to weaning. Rumen morphology and physiology development, microbiota composition, blood metabolites and growth performance. We believe that a greater understanding of these adaptation processes can, ultimately, improve our handling of goat kids at weaning. Recent studies have reported the effects of weaning on the physiological development of ruminants, mainly calves (Diao et al., 2019; Yuste Fernández et al., 2020; Costa et al., 2021). But at the same time it has been observed through different studies, individual variability in growth, consumption and ruminal development due to weaning (Neave et al., 2019). Taking into account the little research that has been carried out using the goat as a study model (Meale et al., 2017), scaled growth over the last decade in the dairy goat sector (Lu and Miller, 2019) and the challenges faced by Quebec producers, we consider that research in weaning age effects becomes relevant (Oudshoorn et al., 2016; Naud and Vasseur, 2021; Naud et al., 2021). For this reason, we hypothesized that late weaning age improves growth performance, rumen development and ruminal microbiota in kids fed with *ad libitum* milk replacer.

In this study, we were able to demonstrate advantages in BW at weaning and ADG during postweaning by delaying weaning age in 8 weeks (18.15 kg ± 6.2 kg, 240 g day^{-1}) and 10 weeks (22.73 ± 6.2 kg, 350 g day^{-1}), exceeding the target weaning weight (10 kg of BW with daily weight gains of 150 g day^{-1}; Hart and Delaney, 2016). On the other hand, we observed that limiting the milk offer by an early weaning, limited the growth potential of goat kids. Although, BW at weaning for EW animals was 13.53 kg ± 6.2 kg, 240 g day^{-1}, we did not observe a compensatory growth regarding to MW and LW in the post-weaning weeks. Similar to our results, studies related to weaning age in calves showed that animals weaned at 6 weeks decrease daily weight gains (Eckert et al., 2015; Meale et al., 2015) compared to animals weaned in a week or two weeks later.

Studies in calves generally offer milk replacer in 8L of milk / day (Khan et al., 2007). Contrary to this, we offered milk replacer *ad libitum* because producers during breeding season, host more than one animal per pen, therefore, it is not possible to follow intake per animal. According to treatments design (weaning age), ME intake from feed changed by day throughout the weaning

phase, since nutritional requirements for kids increased with age. Offering large quantities of milk can be a disadvantage since goat kids has less incentive to include solid feed, as reported in calves (Khan et al., 2011). Similarly, in our experiment, the decrease in ADG during weaning was due to the effect of milk withdrawal, in addition to the low starter and hay intake. Two weeks before weaning, and two weeks after full weaning, it is a critical period for the animal performance. How it was evidenced with the marked contrast in body weights between early and late weaned animals. To mitigate negative effects in animal welfare, milk offer was reduced by 12.5 % everyday for seven days. There are many practices used in dairy operations to limit milk consumption (Eckert et al., 2015; Hart and Delaney, 2016; Zobel et al., 2020) and stimulate solid feed intake. Step down programs have been shown positive effects on behavior, weight gains and an increase in ME intake during weaning (Mirzaei et al., 2020; Mirzaei et al., 2015) by the higher nutrient availability during this stressful period. Although, weaning practices vary from one study to another, it is important to identify the best milk withdrawal technique that is most convenient to the animal and to the producer's economy.

Starch fermentation is indispensable for ruminal papillae development. However, offering granulated starters can cause ruminal acidosis due to high fermentation rates. In this study, we offered *ad libitum* hay and granulated starter at two weeks of age. Available literature on the role of solid feed and feeding management in young ruminants, suggest that performance of ruminants around weaning is also influenced by the physical form of the starter (Khan et al., 2016). A recent study by Li et al., (2021) indicated that relative to lambs fed a granulated starter, lambs fed with a textured starter exhibited better rumen development, nutrient digestibility, and growth performance. Textured starter will cause chewing, which will reduce particles size for further fermentation. In addition, it will increase the production of saliva that serves as a buffer and avoids an acidic environment in the rumen. In the present study, we did not observe animals with signs of acidosis or ulcerated rumens, possibly because we offered high-fiber hay that helped buffer the rapid fermentation of the granulated starter. On the other hand, the *ad libitum* forage possibly was not an absolute advantage because kids could undergo rumen fill. Since forage has a slow fermentation rate and low digestibility. This could explain the preference for milk over solid feed in preweaning for early and medium weaned kids. Late-weaned animals, conversely,

progressively included hay and starter before weaning. This was due to the fact that the animals were larger and were possibly forced to include more nutrients in the diet. Perhaps, giving a more palatable diet may stimulate higher intakes in the first weeks of life, which would accelerate ruminal development. Oudshoorn et al. (2016) conducted a survey with dairy goat producers in Ontario where the variation in the nutritional management strategies between herds was demonstrated. Also, Naud et al. (2021) conducted a study showing variation in performance and production among Canadian dairy goats herds. One of the reasons for the differences between herds could be that there are no set diets for dairy goats at different stages of production. Hence, we believe that more research is needed on solid feed selection. The same as studies in palatability, effective fiber, and feedstuffs with readily degradable carbohydrates that can support rumen development and improve growth performance in dairy goat herds around weaning.

Given the stark differences in nutrient composition intake between one period to another, the transition from milk replacer to solid feed represents a drastic change metabolic profiles of animals (Baldwin et al., 2004). We found that while we were delaying weaning, BHB levels decreased and became stable during the post weaning period, the same as NEFA concentrations. We associate these metabolites variations in blood with increased nutrients intake from solid feed. Animas in EW had a sharp increase of blood NEFA at weaning compared to MW and LW (P< .05). Accordingly, the elevated BHB concentrations in EW animals did not possibly come from ruminal fermentation, but rather from increased supply of NEFA into the liver. Then, change in diet influenced NEFA mobilization by the lack of energy input from milk that varied between treatments, these differences likely were a reflect of stress response induced by the weaning itself. Likewise, a faster energy recovery was observed after weaning in MW and LW. Previous studies in different species have demonstrated the effect of fasting period on energy metabolites. Low energy intake induces catabolic reactions, such as triacylglyerol hydrolysis into NEFA, which can then serve directly as energy substrate. In addition, increased flux of NEFA to the liver increases hepatic fatty acid β-oxidation, that contribute to the synthesis of ketone bodies such as BHB (Geisler et al., 2019). This suggests that, when the animals are in milk for a longer period,10 wk, energy balance is improved, and the transition is less drastic in terms of mobilization of

adipose tissue. Therefore, we speculate that low levels of BHB in LW, respect EW and MW during weaning week were derived from ruminal fermentation, due to solid feed intake at that age. Since BHB concentrations were constant during post-weaning. On the other hand, the ruminal metabolic activity mainly in EW was not sufficient to increase the nutrient supplement to compensate for the decrease in those offered by the milk replacer, which was reflected in negative energy balance at weaning. Therefore, as previously reported, BHB in this study was a useful tool to monitor rumen development, since BHB in the rumen is the product of ketogenic metabolism by rumen epithelial cells (Górka et al., 2018).

Ruminal development begins when solid feed is included in the young ruminant diet, especially a high-carbohydrate diet. In turn, this will stimulate microbial colonization and ruminal fermentation (Khan et al., 2016; Melo and Ungerfeld, 2016). As the ruminant grows, the consumption of solid feed (hay and concentrate) increases, and at the same time stimulates rumen weight, musculature, and epithelial development. At week 12, males were euthanized, to evaluate the state of physical and microbial development of the rumen in the three treatment groups. The whole bacteria community and diversity did not differ among treatments, suggesting that ruminal colonization occurs faster and is directly linked to the offer of solid feed. Therefore, time in milk did not generate an effect on the ruminal microbiota while the animal had access to the solid feed as it was offered in the current experiment. Surprisingly, we observed differences in the microbial community between fungi, protozoa, and eubacteria. Available literature has demonstrated bacteria predation by rumen protozoa activity (Newbold et al., 2015; Belanche et al., 2020) and high-fiber diets are the main contributors of the abundance of fungi community (Belanche et al., 2012). According to this, while weaning age is delayed, the abundance of eubacteria is increased, but in our study, protozoa and fungi were also decreased significantly ($P < .05$). Goat kids in MW and LW had less time to consume fiber from weaning until 12 weeks, when they were euthanized, hence, we speculate that this may explain the low abundance of fungi and protozoa. As a result of bacteria defaunation by protozoa, the proteosynthesis decreases the duodenal flow of microbial protein (Newbold and Ramos, 2020) and it could possibly contribute to lower metabolizable protein intake used by the kid for growth in EW and MW.

Rumen physical development was evaluated at week 12. Animals in LW showed a significantly lighter digestive system and empty rumen compared to MW and EW. The changes observed in the development of rumen tissue are related to forages intake which is the primary stimulators of rumen musculature development and rumen volume (Kosiorowska et al., 2011). Time between weaning and euthanasia allowed the animals in EW and MW to have a longer period consuming hay and concentrate. Feed physical structure probably had the greatest influence on development of rumen muscularization and rumen volume in EW and MW kids. On the other hand, the offer of highly fermentable diets, such as concentrate, contributes to the production of VFAs and therefore jump the rumen epithelial development (Baldwin et al., 2004). Direct action of VFAs on papillae development could not be determined in this experiment however the morphological characteristics of papillae were analysed.

In rumen sacs, papillae differed independently of the treatments. In accordance with Carballo et al., (2019) the papillae heterogeneity in different sacs of the rumen is due to the variety of feeds ingested by a ruminant under grazing conditions. However, the type of hay offer to the kids was always the same, therefore, the differences in rumen histomorphometry observed in the current study could be possible inherent morphological adaptations of ruminants without any possible association with feeding. Total rumen surface area analysis was performed considering width and length in the whole rumen. Results show that LW had greater papillae width compared to MW and EW. We associate this increase in papillae width, with the higher concentrate intake of LW (6 762 g) animal had compared with MW (6 253 g) and EW (6 582 g).

We used lean and fat mass deposition as the ultimate indicators of weaning performance by using DEXA. Previous studies have shown that the effect of carcass composition is related to nutritional level and not to weaning

(Deng et al., 2018; Mao et al., 2019). However, we found that LW presented an advantage in lean mass and fat mass tissue. These, along with NEFAs and BW results, indicate that during weaning, animals did not have adipose tissue mobilization or any decrease in protein deposition that affected the body composition in postweaning, as it was observed in MW and EW. Animals in EW and MW resulted in a lower total mass (kg) at week 12 compared with LW animals (17.4 vs. 18.2 vs. 20.6 kg, $P < .05$). Therefore, we believe that feed intake (high protein and high fat content) as milk replacer, for a longer period, in addition to hay and concentrate offer in preweaning, decreases the weaning-induced negative effects on BW during postweaning period.

We were able to demonstrate that it is possible to have better growth performance by a better adaptation to feedstuffs in preweaning and postweaning, with animals fed milk replacer during the first 10 weeks of life. Also, this work showed that late weaning of kids improved morphological and functional development of the rumen. In addition, BHBA concentrations may be an indicator of intraruminal uptake and metabolism of VFAs, despite a lower rumen volume in LW animals and similarity in rumen microbiota with EW and MW. In our research, we propose late weaning as an alternative that reduces physiological trauma and brings animals to post-weaning with an efficient rumen. In this way, we could offset the costs associated with feed, with costs associated with health and mortality.

Producers focus on costs and not in production. The main disadvantage of delaying weaning in goat kids is mainly due to the costs associated with milk replacer. The economic costs in a dairy herd are the main parameter for the selection of production management practices (Hart and Delaney, 2016; Lu and Miller, 2019). According to Naud et al., (2021), ensuring an environment and nutrition that minimizes disorders and respiratory treatments is fundamental. Each respiratory occurrence has been correlated to low milk production and performance as the goat kid enters in adulthood. Therefore, economic investment in early life in terms of milk replacer offer to goat kids is important to reduce farm losses. The results of this study could contribute to the development of new production parameters such as artificial rearing management option, in which producers could invest in a better replacement animal in dairy goat farms.

3.1 Potential limitations and future perspectives

Due to the study's limitations, caution should be given to interpreting and generalizing these findings to make practical suggestions. Furthermore, some results in this study shed light on future areas in which more research is needed in dairy goats.

One of the limitations of the study was the inability to acquire microbiota samples during weaning in the different treatments through fistulas in kids. Generally, fistulation is an essential tool that allows to access different locations of the rumen to evaluate rumen fermentation or health status. (Castillo and Hernández, 2021). Although, the wide use of cannulation is based on the fact that there is no influence on ruminal function. There is evidence that cannulation can impact microbial rumen communities and animal behavior (HIDARI, 1984; Wang et al., 2018). Assessing the microbial community at weaning would have given us a perspective on the age-related ruminal activity of the animals. Studies related to ruminal microbiota, have shown that rumen microbial development is a temporal and successional process, with age and feeding type playing a significant role (Jami et al., 2013). Consequently, future studies assessing the effect of weaning on rumen morphology in kids should include samples from the different ruminal sacs to get a representative description of rumen development. However, at week 12, we performed analysis of the ruminal content of euthanized animals by sequencing the 16S rRNA gene with Illumina platform. In this way, we were able to observe that, regardless of the weaning age, there were no significant differences in postweaning communities.

On the other hand, metabolic profiles, rumen morphological development, and microbial community are linked and affected by weaning age (Wang et al., 2019; Leal et al., 2021). However, another limitation of our study was the impossibility of correlating these characteristics during weaning. Since samples of VFAs, microbiota, and ruminal epithelium were taken in the postweaning period, 12w. VFAs transport capacity and production increase in the rumen, is a response to changes in diet (Poe et al., 1971). Furthermore, the concentrations of VFAs change dramatically with the passage of time following a meal. Hence, should be interpreted with caution inferences about rumen development and health, by using VFAs and pH data in rumen fluid. Studies in lambs and calves have shown that the increased production of VFAs in the rumen can affect their absorption by the rumen epithelium, and ultimately the capacity of the epithelial cells

to produce ketone bodies (Carballo et al., 2019). Also, the decreased absorption of VFAs is because the ruminal epithelium is not sufficiently developed. Being aware of this limitation, weekly BHB measurements in the blood, were associated with increased metabolic of rumen development, as indicated by greater BHB levels. Similarly, the concentrations of NEFA in blood confirms whether BHB comes from liver or ruminal ketogenesis. (Chapter 2).

To evaluate the weaning effect on carcass composition at week 12, DEXA tool was used. However, DEXA results should be interpreted with caution considering that we did not perform in vivo scanning. Since it would have affected the animal behavior by the displacement and the intervention with sedatives. To address these results, we used body weight and NEFA data that were taken weekly. According to animal studies, DEXA requires calibration protocols to obtain accurate data (Pomar et al., 2017; Calnan et al., 2021). We believe that more research is needed in the use of DEXA at different points of time, to properly assess the effect of age on the body composition of kids.

Although, dairy goat production is characterized by different advantages over dairy cow production, which includes, a short reproductive cycle, market niche and fast economic return. Goat production continues to have many challenges at the first base of the production chain, starting with the few breeds with high dairy production, no established rearing techniques, and the few large-scale production farms mainly in the province of Quebec. Regarding weaning, research is needed on future effects on the different weaning methods. We followed kids until week 12, and the effects on weight after weaning were evident for the animals weaned early compared to the other treatments. Similarly, post-weaning follow-up in LW animals was very short. Therefore, the future performance of these animals in a longer state is uncertain, since the animals continue to face different challenges related to management, such as, regrouping, coupling and diet change. For this, epigenetic studies would be a useful alternative, together with long-term follow-up, to identify reliable transgenerational biomarkers, related to heritable disorders, in the often-overlooked areas of livestock, such as immunity and stress. Considering that, in tissue and cells, the epigenome responds in a specific way to internal and external environmental signals (nutrition, pathogens, maternal behavior, climatic conditions, weaning,

management practices, etc.) (Anderson et al., 2012; Triantaphyllopoulos et al., 2016; Wang and Ibeagha-Awemu, 2021).

3.2 Overall conclusions and implications

The results of our study support recommendations for practices such as weaning time in dairy goat operations that should be employed to set kids up for a more successful weaning, in terms of performance and rumen development. Feeding with *ad libitum* milk for 10 weeks, allows kids greater ME intake to reach their preweaning growth potentials, without sacrificing long-term rumen development. Additionally, to encourage greater solid feed consumption and potentially a better weaning transition when feeding high levels of milk, solid feed should be included *ad libitum* since week 2. Utilizing a gradual weaning program, by reducing milk in 12.5% for one week, can also help encourage feed intake early in life. Quality goat kids and later quality dairy goats by prolong milk offer for 10 weeks to meet herd potential is possible. With the advantages demonstrated in weight at weaning by animals weaned late, is expected to detect high reproductive performance and high milk yields in the adulthood, as it was demonstrated in previous studies with calves (Soberon and Van Amburgh, 2013)

Overall, dairy goat producers should consider all aspects of nutritional programs and management when developing optimal kid rearing protocols to help ease kids through the stressful weaning transition. This allows producers to invest in better animals, with better growth rates and health.

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