Trabajo de Fin de Máster

Título: Determination of amino acids potentially limiting the growth of milk fed calves

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Abstract

Determination of amino acids potentially limiting the growth of milk fed calves

The objective of this study was to compare the effect of feeding milk-replacer (MR) with or without added specific amino acids on calf growth and plasma amino acids concentration. Eighty calves were selected at random from a commercial farm. The average age at entry was 2.8 ± 1.75 d and the average initial body weight was 43.5 ± 6.36 kg. Calves were distributed to 1 of 4 MR treatments: 1) Control, a MR with 24.5% CP and 21.0% fat; LMT treatment, the MR supplemented with 0.62% L-Lys, 0.22% L-Met and 0.61% Thr; 3) PT treatment, the MR supplemented with 0.2 % Phe and 0.2 % Tyr; and PG treatment, the MR supplemented with 0.3% Pro and 0.1% Gly. All treatments were fed daily for 56 d. Calves received 4 l/day from day 1 to 4, 5 l/day from day 5 to 14, 6 l/day from day 15 to 49 and 3 l/day from day 50 to weaning at 56. Calf starter and straw were offered throughout the study. During this period, feed intake and growth performance were measured. There were no differences among treatments in body weight (BW), wither height (WH), average daily gain (ADG) or feed efficiency. From day, 1 to weaning at day 56 DM intake was in average 61.8 kg, ADG 787.3 g/d and feed efficiency 0.64. At weaning, average BW, WH and BW: WH ratio was 88.9 kg, 98 cm and 0.91, respectively. Therefore, feeding calves with an MR that contains 24.5% CP and 17% fat contains sufficient amino acids to support the target growth.

Keywords: calves, milk replacer, amino acids, growth performance.

Resumen

Determinación de los amino ácidos potencialmente limitantes en el crecimiento de los terneros lactantes

El objetivo de este estudio fue comparar el efecto de la alimentación de lacto reemplazante (LR) suplementado con aminoácidos específicos sobre el crecimiento de terneros y la concentración de aminoácidos plasmáticos. Ochenta terneros fueron seleccionados al azar de una granja comercial. La edad media a la entrada fue de 2,8 ± 1,75 d y el peso promedio inicial fue de 43,5 ± 6,36 kg. Los terneros se distribuyeron en 1 de 4 tratamientos de LR: 1) Control, LR con 24,5% de CP y 21,0% de grasa; LMT tratamiento, el LR complementado con 0,62% L-Lys, 0,22% L-Met y 0,61% Thr; 3) Tratamiento PT, el LR suplementado con 0,2% de Phe y 0,2% de Tyr; Y PG, el LR suplementado con 0,3% de Pro y 0,1% de Gly. Todos los tratamientos se alimentaron diariamente durante 56 d. Los terneros recibieron 4 l / día desde el día 1 hasta 4, 5 l / día desde el día 5 hasta 14, 6 l / día desde el día 15 hasta 49 y 3 l / día desde el día 50 hasta el destete a los 56. Se ofreció concentrado y paja durante todo el estudio. Durante este período, se midieron el consumo de alimento y el crecimiento. No hubo diferencias entre los tratamientos en peso corporal (BW), altura (WH), ganancia diaria media (ADG) o eficiencia de alimentación. Desde el día 1 hasta el destete al día 56 la ingesta de materia seca fue en promedio de 61,8 kg, ADG 787,3 g / d y la eficiencia de la alimentación 0,64. Al destete, el promedio de BW, WH y BW: WH ratio fue de 88,9 kg, 98 cm y 0.91, respectivamente. Por lo tanto, la alimentación de terneros con un LR que contiene 24,5% de CP y 17% de grasa contiene suficientes aminoácidos para apoyar el crecimiento deseado.

Palabras clave: terneros, lacto reemplazante, aminoácidos, crecimiento.

Résumé

Determmination des acides amines potentiellement limitants pour la croissance des veaux allaitants

L'objectif de cette étude était de comparer l'effet de la supplementation des quatre traitements du lait reconstitue (LR) avec des acides aminés spécifiques sur la croissance des veaux et sur la concentration d'acides aminés plasmatiques. 80 veaux ont été sélectionnés au hasard. L'âge moyen à l'entrée était de 2,8 ± 1,75 d et un poids corporel initial moyen de 43,5 ± 6,36 kg, pesé et attribué à recevoir 1 des 4 traitements. Le traitement contrôle était LR avec 24,5% de CP et 21% de lipides, le traitement LMT était composé de 0,62% de L-Lys, de 0,22% de L-Met et de 0,61% de Thr. Le traitement PT été composé de Phe à 0,2% et 0,2% de Tyr. Le traitement PG été composé de 0,3% de Pro et 0,1% de Gly. Tous les traitements ont été distribués quotidiennement pendant 56 jours, les veaux recevaient 4 l / jour de 1 à 4 jours, 5 l / jour de 5 jours à 14,6 l / jour de 15 à 49 et 3 l par jour du 50 éme jour au sevrage a 56j. Le concentre et la paille ont été offerts tout au long de l'étude (63 jours). Au cours de cette période, l'apport alimentaire et la croissance ont été enregistres. Nos résultats du 1er jour au sevrage (56j) étaient en moyenne de 61,8 kg pour la matière sèche ingérée total, un gain quotidien moyen de 787,3 g / j et une efficience de 0,64. Au sevrage, nous avons noté 88,9 kg, 98 cm et 0.91 respectivement pour PV, la hauteur (H) et le ratio PV: H. En conclusion, il n'y avait pas de différence de poids corporel entre les traitements par l'ajout des AA au cours de la période de pré sevrage. D'autre part, aucune différence notée en ce qui concerne le gain moyen quotidien et l'efficience alimentaire entre différents traitements. Par conséquent, l'alimentation des veaux avec LR contenant 24,5% de CP et de 17% de matières grasses contient suffisants d'acides aminés pour soutenir la croissance souhaitée.

Mots-clés: veaux, lait reconstitue, acides aminés, performance de croissance.

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List of abbreviations

AA: Amino acid

ADF: Acid detergent fiber

ADG: Average daily gain

aDMI: Average dry matter intake

BW: Body weight

CF: Crude fiber

CI: Conversion index

CP: Crude protein

DM: Dry matter

DMI: Dry matter intake

Ef: Efficiency

Gly: Glycine

IU/kg DM: International unit per kg of dry matter

LMT: Lysine, Methionine and Threonine

MR: Milk replacer

NDF: Neutral detergent fiber

NEAA: No essential amino acids

NRC: National Research Council

PDI: Intestinal digestible protein

PG: Proline and Glycine

Ph: Phenylalanine

PT: Phenylalanine and Tyrosine

SMP: Skim milk powder

UFL: Unit forage milk

VFA: Volatile fatty acids

WPC: Whey protein concentrate

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Introduction

1. Introduction

The nutrition of lactating calf is critical because it is related to its health status that has a very important effect on the speed of growth, especially during the first 6 months of life (Donovan et al., 1998). An adequate growth model makes it possible to better express the genetic potential of the future producers, namely the determination of age at first insemination, age and weight at the first calving and hence the production of milk for heifers and a good conversion index for fattening calves (Heinrichs, 1993).

During the first 1 or 2 months of life, the digestive physiology of calves is like that of mono-gastric animals. At about 2 months of age, it begins to function as that of adult ruminants (Radostits et al., 2000). The provision of adequate amounts of high-quality of colostrum at birth, sufficient milk and / or milk replacer (MR), optimum weaning and adequate dry feed intake are all factors of great importance for overall well-being and growth of calf.

Commonly, exist two methods of feeding MR; conventional feeding program of calves which consists on feeding 4 l/d of milk replacer (MR) at a concentration of 12.5% DM from 3 to 60 d of age to attain ADG around 500 g/d, to promote an early consumption of calf starter and to achieve a good rumen development while decreasing nutrition costs (Davis and Drackley, 1998), and accelerated feeding program to get ADG around 900 g/d.

Another way to improve calf's performance is to supplement MR with AA especially those called essential such as Lysine and Methionine. This supplementation has demonstrated its proof in several studies. Kanjanapruthipong, (1998) reported that feeding MR (concentrated at 13% DM and 21% CP) containing soy protein and supplemented with AA presented a greater ADG and N retention than calves receiving an MR without AA supplementation. However, rare are the articles that deal with NEAA such as Proline and Glycine concerning calf's growth despite their importance in life cycle of calves.

Therefore, the objective of this study was to compare the effect of offering four milk treatments with or without added amino acids to compare calf growth and plasma amino acids concentration. Our hypothesis was that specific AA (Lys Met and Thr, Ph and Tyr, Pro and Gly) would be limiting ADG in calve of 8 weeks age.

Literature review

2. Literature review

2.1. Liquid feeding for calves

2.1.1. Colostrum

Colostrum is the first milk secretion after calving. In addition to its very high nutritional value, colostrum contains three essentials types of immunoglobulin: IgG (80-85%) and IgM (7-10%) that are absorbed at the intestinal level and provide systemic protection against infectious agents, and IgA (7-10%) that are larger, and can't be absorbed at the intestinal level, but provide a local protection in the intestine, which is an important aspect in the prevention of digestive problems**.**

This passive transfer of immunity is of great importance for calves on the one hand because the placenta barrier does not allow the transplacental passage of immune-globulins, resulting in a very vulnerable calf; and on the other hand, if the quantity transferred is insufficient occurs what is called the failure of the passive transfer of the immunity, which affects 20-40% of calves in the US and Canada (Beam et *al*, 2009) and is responsible for 40-50% of mortality during the2-3 first months of life (Margerinson and Downey, 2005). Several factors influence the concentration of immunoglobulin in colostrum such as the breed, age, the quantity of colostrum produced (Besser et *al*., 1991) and the time between calving and the obtaintion of colostrums.The time between calving and collection of colostrumis inversely proportional to its quality so, it will be best if colostrums are collected within two hours of the postpartum (as it increases postpartum time, reduces the concentration of IgG (Chigerwe et *al*., 2008). The passive transfer of immunoglobulins from colostrum does not have a direct effect on growth but influences weight and height, through its effects on the health of the calf (Heinrichs, 2007).

Partial or complete failure in the passive transfer of calostral immuno-globulins is the main factor responsible for neonatal diseases and mortality in calves.

2.1.2. Whole milk

Once colostrum is fed, whole milk is the natural liquid feeding for calves. It is nutritionally balanced; it contains an excellent protein source and high digestible fat (Table 1). However, it is common to feed MR, because they are cheaper. For example, feeding 4 l/d whole milk costs 1.32 €/d in contrast to the 500 g/d of MR powder that costs 0.72 €/d (Terre et al., 2007). Others alternative cheap sources of feeding calves is to use transition and waste milk. However, feeding waste milk is dangerous because of its contamination with antibiotic and mastitis. When waste milk is fed, it is recommended pasteurizer it to avoid diseases transmission (Kesler et *al*., 1981). Discarded milk improved calf growth performance, and decreased mortality risk compared with calves fed a conventional MR (Godden et *al*., 2005). The only problem associated with feeding wasted milk, is the development of antibiotics resistance. Selim and Cullor (1997) observed antibiotic-resistance in calves fed discarded milk. Therefore, it is strongly advised not to feed milk of mastitis or containing antibiotics in order to limit the problems of resistance and imbalance of the intestinal flora (Serieys, 2009). Nevertheless, the use of this non-marketable milk represents a significant economic loss in many farms (Brunschwig, 2009).

Table 1. Average nutritional composition of whole cow's milk (per 100 g; usda, 2007).

CP (g)	Fat (g)	Lactose (g)	Ash (g)	Ca (mg)	P (mg)	Energy (kcal)
3.2	3.9	4.8	0.69	113	132	60

Whole milk should be hot (about 40ºC) twice daily at regular intervals, not exceeding 3l per meal. Dilution of milk with water should also be avoided. Although this feeding system seems to present critical points for the health of calves, it is still widely used by breeders. Indeed, with this type of breastfeeding, the breeder can use his discarded milk and manage a surplus of quota. Two examples of a whole milk feeding plan are presented in Tables 2 and 3.

Table 2. Whole milk feeding plan (weaning at 10 wk; Arzul and Besnier, 2007).

<table>
<tr><th>Age (Wk)</th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th><th>9</th><th>10</th></tr>
<tr><td>Number of meals</td><td colspan="7">2</td><td colspan="3">1</td></tr>
<tr><td>Amount of milk l/d</td><td>4</td><td colspan="6">6</td><td>3</td><td colspan="2">2</td></tr>
</table>

Table 3. Whole milk feeding plan (weaning at 14 wk; Arzul and Besnier, 2007).

Age(Wk)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Number of meals	2			1										
Amount of milk l/d	4 to 6	6 to 7	7	4.5								3	2	

2.1.3. Milk replacer

Today there is a wide range of MR on the market, which responds to different systems of calf rearing, in order to promote better performance of the animal and the economy. Milk replacers can be classified by protein source, protein/fat ratio and amounts of medication or additives added.

Protein levels in calf milk replacers range from 18% to 30% crude protein (CP). Calf growth and performance are related to a large number of factors, among these we find: protein levels, protein/energy ratios, and protein sources interact. Growth is regulated by daily intake of protein and energy; therefore, different feeding levels will also affect performance (BAMN Publication, 2008).

- **Protein content**

The MR is classified based on the amount and source of protein (Table 4).

The no milk proteins have variable effects on calves' performance. In general, the greater the substitution of milk protein, the less the performance of calves, because it depends on the nature and the amount of replacement protein; also it depends on the amino acids supplementation.

Kaiwu (2015) used four sources of plant proteins (soybean, wheat, peanut protein, and rice protein) vs. full milk protein. Soybean protein and rice protein have given results similar to those of milk protein concerning ADG and feed: gain ratio. However, wheat and peanut protein have given worse effects.

Kanjanapruthipong (1998), reported that alternative vegetable protein such as 43% soybean meal, in MR, decrease body weight gain from 388g for milk replacer with skim milk powder to 244.3g, due to lower digestibility and absorption of AA. In addition, vegetable protein decreased the apparent digestibility of MR, for example, appearent digestibility of methionine equal to 92 for milk replacer with skim milk powder protein vs. 77.1 for milk replacer with soybean protein, because of their anti-nutritional factors (Montagne and *al.*, 2001).

Table 4. Origin of milk replacer protein (adapted from BAMN Publication, 2008)

Milk Protein	Alternative Protein
Dried Whey Protein Concentrate	Soy Protein Isolate
Dried Whey	Protein Modified Soy Flour
Dried Whey Product	Soy Protein Concentrate
Skim Milk	Soy Flour
Casein	Animal Plasma, red blood cells
Sodium or Calcium Caseniate	Wheat gluten or isolate
	liquid egg, spray-dried whole egg
	Fish protein

- **Fat content**

There are also different fat sources to substitute milk fat, with the objective of reducing the cost of production. For example, tallow, lard or vegetable oils (coconut oil, palm oil) as fat MR source. Fat levels vary between 10% and 28%, but the most common percentage added range from 18 to 22%. Fat is the most important energy source. It is responsible of the difference in energy levels and metabolizable energy content of milk replacers because it is primarily determined by the fat and carbohydrate levels. Therefore, fat sources should be highly digestible to the young calf and preserved with an antioxidant to prevent rancidity (BAMN Publication, 2008).

- **Carbohydrate source**

Carbohydrates are an important source of energy for growing calves. In most MR, lactose is the main source of carbohydrates because of calf's lactase activity and consequently, glucose and galactose can be effectively utilized in large amounts by calves (Davis and Drackley, 1998).

Lactose is not only a good source of energy, but it also aids in the absorption of magnesium, calcium, zinc and iron.

- **Additives**

Additives can be used to improve industrial processing and preservation of MR such as antioxidants (tocopherol which is a form of Vitamin E), stabilizers (lecithin, pectins), or preservative (citric acid). The NRC (2001) recommendations for vitamins are lower than the amounts found in whole milk, and those used in the industry (Table 5). In contrast, trace minerals are added in greater amounts than in whole milk to avoid deficiencies.

Table 5. Requirements of milk vitamins for calves.

Vitamins	Roles	Whole Milk IU/kg DM	NRC,2001 IU/kg DM	Manufacture
Vit. A	necessary for normal growth development	11,5000	9,000	25,000 40,000
Vit. E	Supports immune function and protects cellular membrane from oxidative damage	8	50	65 – 85
Vit. D	maintains calcium and phosphorus homeostasis	300	600	4,000 10,000

In addition, the procuration of antibiotics to enhance growth was used in formulation of MR, but its addition increase also antimicrobial resistance; for that reason, EU has forbidden the use of antibiotics as growth promoters in animal nutrition. This has prompted the industry to look for alternatives to antibiotics such as the supplementation. Lactoferrin is a 78-kDa iron-binding protein that is an effective antimicrobial agent against a wide range of infectious, such as *Escherichiacoli* (Reiter and Perraudin, 1998), *Salmonella* spp., and *Staphylococcus* spp. (Weinberg, 2001), which demonstrated that increased BW, ADG, stimulated calves to eat more dry feed and, increased feed efficiency compared to calves fed MR without lactoferrin (Joslin and *al*., 2002). However, Cowles and *al*. (2006) reported that lactoferrin supplementation in MR fed to pre weaned dairy heifers had no effects differences. Bentonite, which is natural clay, has shown its effectiveness in preventing diarrhea in calves prophylactically for 6 days (Bartos and Habrda, 1974).

Proper preparation of milk is recommended because the main causes of diarrhea are related to poor preparation of breastfeeding foods. Some rules are strictly to respect:

- adequate dosage to limit any digestive problems;
- Milk reconstitution water must be bacteriologically sound;
- The preparation of the emulsion with water at a temperature of 50 to 58°C and the appropriate distribution temperature is around 40°C.

Two examples of milk powder (concentration = 130 g/l) breastfeeding plans are presented in tables six and seven.

Table 6. Feeding plan for milk replacer (Radigue and Hoelgen, 2006).

Age (Wk)	1	2	3	4	5	6	7	8	9	10
Number of meals	2									
Amount of milk /meal (l)	1.5	2	2.5	3				2.5	2	
Amount of milkreplacer/meal (g)	195	260	325	390				325	260	

Table 7. Feeding plan for milk replacer (Radigue and Hoelgen, 2006).

Age (Wk)	1	2	3	4	5	6	7	8	9
Number of meals	2				1				
Concentration (g/l)	140				200				150
Amount of milk /meal (l)	1.5	2	2.5	3	3.5			2.5	2
Amount of milk replacer/meal (g)	210	280	350	420	700			500	300

2.1.4. Weaning

At birth, the calf functions like a monogastric and it is prepared to digest milk. In fact, the abomasum is larger than the other gastric compartments, being on average 2 liters capacity versus 0.75 liters for the reticulo-rumen **(**Alves De Oliveira and Bezille, 2006).

Weaning is the transition period from liquid to solid feeding, calves should be weaned when they are able to grow on a diet without milk. To make it possible, calves should be already eating at least 1 kg of starter.

Different opinions on the time of weaning; there are authors who say that calves should eat on average 2 kg/d of starter, others like Drackely (2008) said that calves should not be weaned until they are consistently eating 1 kg of starter daily; because starter is as an essential promoter of rumen development. A smooth transition from liquid feed (milk or milk replacer) to solid feed (grains or forage) is important in minimizing weight loss and distress at weaning (Weary et *al*., 2009). During this period, the distribution of milk is reduced and then suppressed (on the day of weaning; Roy, 1980), while the quantity of solid food ingested increases consequently. Then the rumen will take more importance at the expense of the abomasum, followed by changes in the digestive and metabolic function that will allow animals to adapt to the ingestion and use of solid foods.

- **Changes at weaning**

As MR has been fed to calves, pre weaning ADG has increased and pre weaning starter intake has decreased (Jasper and Weary, 2002; Cowles et *al*., 2006; Hill et *al*., 2013). However, when the DM fed from liquid was more than 0.7 kg of DM and weaning has taken place, the live weight gains decreased as the milk was reduced or suppressed, but it increased one or 2 weeks after weaning (Hill et al., 2007, 2013, 2016). DM digestibility decreased immediately after weaning compared with calves of the same age that were still nursing (Funaba and *al.*, 1994), probably because milk is more digestible than starter (Terre et al., 2007). But, increased progressively during the post weaning period (Funaba and *al*., 1997), to compensate the low DM digestibility (Funaba and *al*, 1994).

2.2. Solid feeding of calves

2.2.1. Starter

The starter concentrates contribute to the development of the rumen by the production of volatile fatty acids (VFA), in particular butyrate and propionate, which are derived from ruminal fermentations and which stimulate rumen papillae growth (Khan, 2011). In fact, calves receiving only milk, it was observed that the rumen remains thin and smooth and the size of the papillae increases only very little (2 mm in 12 weeks). On the other hand, Allen (2004) found in calves consuming a reasonable amount of cereals from an early age that had more developed papillae (10 mm to 12 weeks; Figure1).

Figure 1. Rumens of 6-Week-Old Calves.

There is a wide variety of starter concentrates in the market. Starter should include:

- An energy source: wheat, barley, triticale, spelled, corn grain or corn breaking, oats, bran, beet pulp, soybean hulls, cotton seed, vegetable fat or extruded seeds;
- A source of protein: soybean, rapeseed, sunflower, copra or palm kernel, alfalfa;
- Minerals, trace elements and vitamins (AD3E in particular).

In addition, starter may include yeast, buffering agents (sodium bicarbonate, magnesium oxide or calcium carbonate), sweeteners and flavorings to enhance dry matter intake. These concentrates exist in different forms: flocculated, granulated or mash (mixture of raw or flattened raw materials). They are usually distributed for calves consuming milk between 1 and 6 weeks of age (Table 8).

Table 8. Nutritive values of starter concentrate for calves (Arzul and Besnier, 2007)

UFL	CP (%)	PDI (g)	CF (%)	Ca (g)	P (g)
0.95	16-18	100-120	8	11	6

In some case, a second-age starter concentrate is also used. They are used in calves of more than 4 to 7 weeks depending if the weaning is rather early or late.

In terms of quantity, it is estimated that the calf should gradually increase calves consumption of concentrates until reaching 2 kg at the time of weaning (minimum 1 kg). We start with 50 to 100 g of first-age starter available per day during the first week of life, increasing progressively up to 1 kg to 2 kg. It should be noted that a dietary transition is required over a week between first age and second age starter. The two essential qualities of a good calf concentrate are palatability and non-acidogenicity (Allen, 2004).

2.2.2. Forage

The consumption of forage by calves promotes the development of the musculature of the rumen wall by a purely mechanical friction effect. However, as with concentrates, forages do not produce sufficient VFA to ensure papillae development (Allen, 2004). In fact, in calf consuming milk with good hay, papillae were less developed and the rumen wall was thinner. Feed ingestion also contributes to the development of ruminal motricity by stimulation of the reflexogenic zone, which favors food catabolism and production of saliva**.** However, forage feeding was discouraged during the milk feeding phase, based on research showing lower starter intake and poorer weight gains in calves provided forage (Stobo et al., 1966).

2.2.3 Water and mineral-vitamin block

Water and salt must be available from the first week of life in order to participate in the development of the microbial flora of the rumen. Nevertheless, access to water should be restricted immediately after the distribution of the dairy food in order not to favor the "ruminal drinker" (Alves De Oliveira and Bezille, 2006). As the age of the calf increases, the amount of water passing through the rumen becomes more and more important and partially compensates for the lack of saliva produced by the calf. Water must be accessible and not contaminated. The proposed water must be of high quality.

2.3. The growth of Holstein calves

Several plans for breastfeeding exist for calves. They differ by the quantity or the nature of the liquid food and the way of distribution (Pirouelle, 2009). Nevertheless, the objectives of the milky phase remain the same:

- Ensure optimal growth, both skeletal and muscular, resulting in an average daily gain of 500 g/d when calves are fed at 10% of their body weight divided into two meals and 900 g/d or more when giving milk or milk replacer *ad libitum(ad libitum* means; when calves consume about 20% BW/d, equivalent to an average of 10 to 12 l of whole milk for Holstein calves (Jasper and Weary, 2002; Khan et *al.*, 2007a; Sweeney et *al.*,2010)**,** or increasing the amount dispensed gradually (Quigley et *al.*, 2006)
- Pursure the transformations of the digestive system, until the weaned animal becomes ruminant (Arzul and Besnier, 2007).

The objective and the orientation of the breeding determine the average daily gain desired (conventional or accelerated), the type of weaning (early or late) and the relative importance given to health or economic returns.

Lower growth leads to insufficient body development, which will subsequently limit the expression of the genetic potential of the animal and reduce the longevity of its career. On the other hand, growth that is too high, that is to say greater than 1000 g/d, can give rise to an overly fat animal (Pirouelle, 2009).

2.3.1. Conventional growth

Conventional feeding program of calves consists on feeding 4 l/d of milk replacer (MR) at a concentration of 12.5% DM from three to 60 d of age to attain ADG around 500 g/d. This feeding program promotes an early consumption of calf starter and achieves a good rumen development with a low cost (Davis and Drackley, 1998). However, growth rates are low (Flower and Weary, 2001), For example, average daily gain for the calves fed *ad libitum* was 0.8 ± 0.10 kg in the first week after birth, compared to 0.2 ± 0.10 kg for the conventionally fed calves (Jasper and Weary, 2002).

In one experiment, it compared conventional milk feeding for calves with an *ad libitum* milk feeding consumption for 42 d; it noted that the conventionally fed calves, achieved significantly higher daily intakes of both starter and hay over the pre weaning period. The conventionally fed calves had consumed an average of 6.11 kg of starter and 0.98 kg of hay. In comparison, calves fed milk *ad libitum* consumed only 2.99 kg of starter and 0.52 kg of hay. However, at weaning and post weaning, intake of starter and hay increased rapidly for both groups, and there were no significant treatment differences over this period (Jasper and Weary, 2002).

Khan (2007)**,** did the same experience of comparing conventional milk program with a STEP method (enhanced program), and found a rapid increase in intake of both starter and hay after 28d in calves fed STEP program, then a pronounced surge in solid feed consumption was observed in both groups during weaning and post weaning (Figure 2; khan et *al*., 2007).

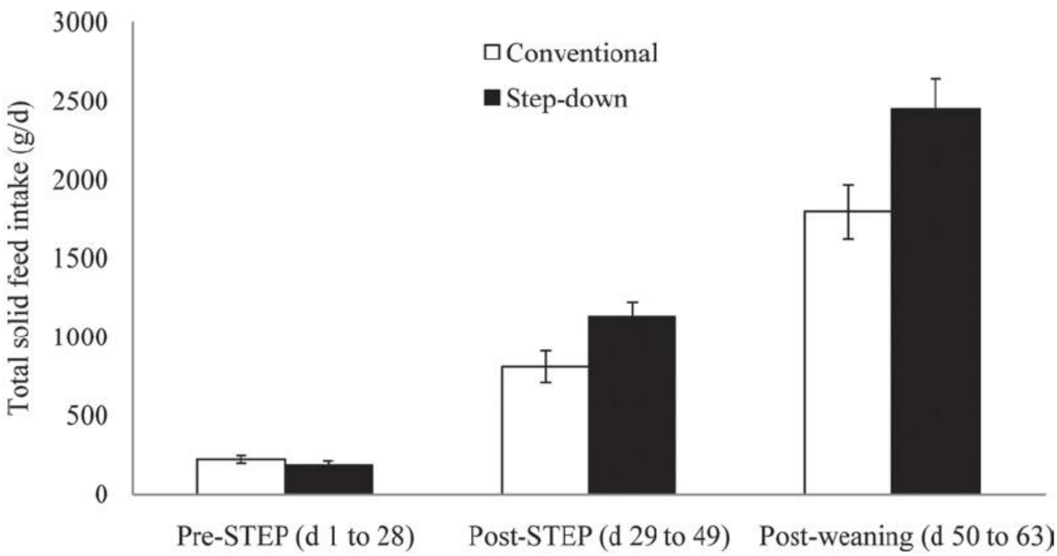

Figure 2. Total solid intake by Holstein calves fed milk replacer through two methods.

Additionally, genetic potential of suckling calves is not fully expressed in conventional feeding programs. This restricting milk rations also seems to reduce play behavior in calves; it has been observed that calves fed 6 l/d ran (which is one of indicator of play behavior) far less than those fed 12 l/d (Krachun et *al.*, 2010).

Khan (2007) concluded that conventional or restricted feed program leave calves hungry and compromise growth, health, welfare, and future milk production.

2.3.2. Accelerated growth

In accelerated growth feeding programs, high levels of MR and crude protein (CP) arerecommended. However, higher intakes affect solid feed consumption and weaning in dairy calves. It delays the initiation of ruminal fermentation and development (Baldwin et *al*., 2004). High amounts of nutrient in the intestinal lumen may prompt intestinal disorders caused by undigested lactose that enters the colon and promotes water accumulation to the intestinal lumen caused by an increase of the osmotic pressure in the colon (Roy, 1980). Therefore, a major fear of feeding MR with high DM concentration is the risk of causing diarrhea by mal digestion. Diarrhea can cause weight loss and dehydration (Pettyjohn et *al*., 1963).Consequently, water consumption may increase to compensate the loss of liquid by the loose feces obtained with high MR concentrated diets. In general, when feeding large amounts of MR, ADG is improved, but solid feed intake decreases (Huber et *al*., 1984).

Consequences of accelerated growth on:

- **Growth performance**

Accelerated growth gives a greater growth rates in record time of 2 or 3 months that reduces the cost of rearing (Kertez et*al*., 1998). However, an inverse relationship between milk and solid feed intake has been demonstrated in several studies (Terré et *al*., 2007; Raeth-Knight et *al*., 2009).

Stamey et *al.* (2012), noted that feeding conventional feeding program (20/20) resulted in greater concentrate intake then accelerated feeding program (28/15), but less ADG comparing with accelerated feeding during pre weaning period (Figure 3; Stamey et al., 2012).

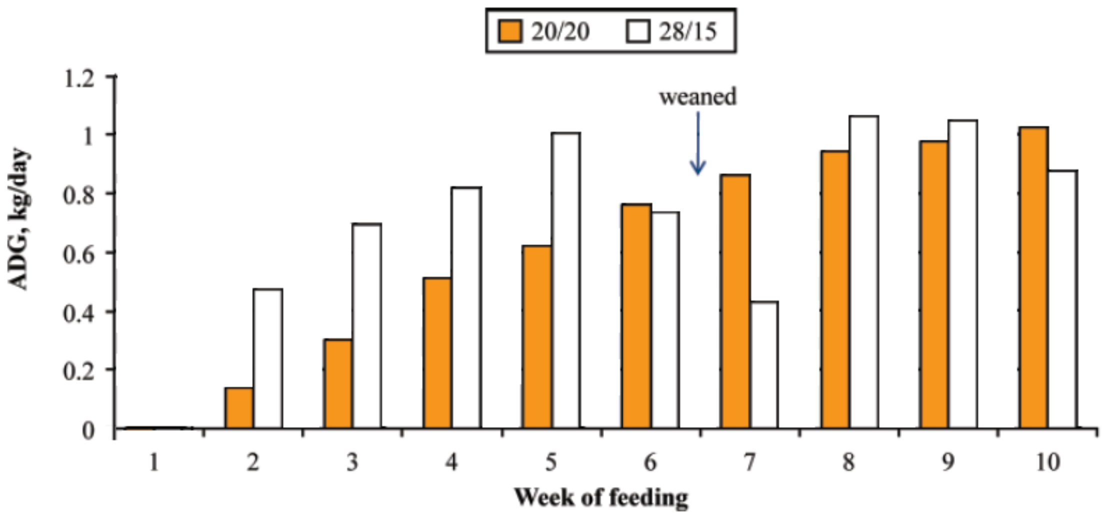

Figure 3. Weekly ADG for 20% CP/20% fat and 28% CP/15% fat milk replacers for pre- and post weaning periods.

- **Nitrogen metabolism**

In calves fed isoenergetic MR with increasing CP levels (from 14% to 26%; Blome et *al*., 2003), or in calves fed an MR with 30% CP at a dilution rate of 15% or 18% (Diaz et *al*., 2001), growth rates and N retention (g/day) increased linearly as the MR protein/energy ratio and the MR dilution rate increased, respectively. However, plasma urea concentration also increased as the protein/energy ratio increased, indicating that calves fed MR at high CP did not utilize dietary N as efficiently as calves fed MR at low CP content (Blome et *al*., 2003).

- **Rumen development**

The calf from monogastric to polygastric should pass through major changes at the gastric level. At birth, abomasum is then preponderant in comparison with the others gastric compartments: on average 2 liters vs. 0.75 liters for reticulo-rumen which corresponds to 60% of the total.In adulthood it is the rumen that grows to the detriment of the other compartments (more than 80%) while the abomasum represents only 8% (Figure 4; Alves De Oliveira and Bezille, 2006). These changes are influenced by the changes in feeding from the liquid feed to the solid feed.

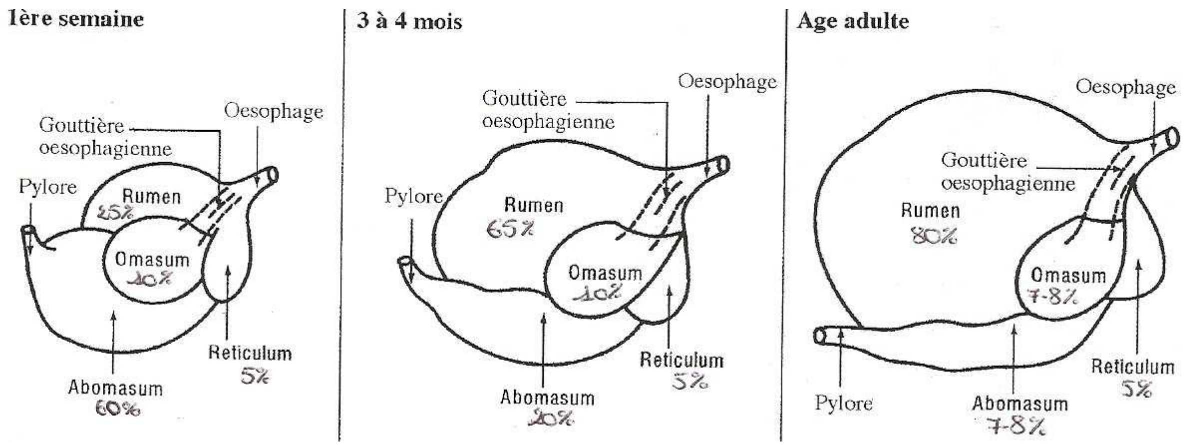

Figure 4. Development of compartments of the stomach of cattle from birth to adulthood.

The rumen of a neonatal calf is incompletely developed physically and nonfunctional metabolically (Warner et *al.*, 1956). The initiation of solid feed intake by the newborn calf; helps the rumen in his physical and metabolic development. The physical development of the rumen consists in the increase of rumen mass and growth of the papillae (Baldwin, 2004).

Calves following an accelerated growth feeding program usually have slower rumen development (Khan et *al.*, 2007a,b) due to low starter intakes during the pre weaning period (Shamay et *al.*, 2005).

In addition, the low starter fed during the pre weaning period in accelerated growth fed calves, may delay rumen microbial development (Jasper and Weary, 2002).

Feeding larger amounts of milk before weaning may delay physical and metabolic rumen development depressing the solid feed consumption around weaning (Hill et *al.*, 2010; Sweeney et *al.*, 2010). In contrast, khan et *al.* (2007b), reported that providing calves a large amounts of milk early in life and then reducing intakes before weaning (step-down method) caused an increase in solid feed consumption (Figure 2). Ruminal development in calves can be affected also, by greater supply of energy and nutrients from higher milk intake which contribute directly to the development of rumen papillae through metabolism (Shen et al., 2004).

2.4. Role of amino acids in calf growth

Proteins are composed of 20 amino acids, many of which calf can synthesize in adequate amounts to meet its needs. But several amino acids must be consumed in the diet because calf cannot synthesize these amino acids are often called “essential” and include lysine, histidine, leucine, isoleucine, valine, methionine, threonine, tryptophan, tyrosine, and phenylalanine. Milk replacers must contain these amino acids in sufficient amounts to meet the needs for maintenance and growth of young calves. Calf performance may be limited by insufficient intakes of certain essential amino acids, particularly lysine and methionine, both amino acids which have been widely studied because they have often proved to be the most limiting for calves growth (Klemesrud et *al*., 2000), and on the other hand because there are forms that are effectively protected against their degradation in the rumen which facilitate their studies (Rulquin et *al*., 2001).

Nowadays, all AA are needed for rapidly growing calves, because of their important roles. Many NEAA (e.g. arginine, glutamine, glutamate, glycine, and proline) and certain EAA (e.g. leucine and tryptophan) participate in cell signaling, gene expression, and metabolic regulation. Thus, functions of AA beyond protein synthesis should be considered in dietary formulations to improve efficiency of nutrient use, growth, development, reproduction, lactation, and well-being in animals (Guoyao, 2014).

Several studies have demonstrated the supplementation effect of MR by essential amino acids, such as lysine, methionine, threonine, tyrosine and phenylalanine. However, there are rare studies that deal with non-essential amino acids regarding growth calves.

Some of these studies are:

Terre (2006) reported that for conventional feeding program calves fed 4 l/day of MR at 12.5% DM; observed that plus phenylalanine and tryptophan are available, plus ADG increases and less plasma urea concentrations, and conclude that phenylalanine and tryptophan could be limiting growth in calves fed conventional feeding programs.

For its part, Hill (2008), compared MR with different concentrations of CP (26%, 28% CP) with Lys (2.34%) and Met (0.72%) and the same MR without AA; noted 16% improvement in ADG for calves fed MR (26%) supplemented with AA then for MR without AA. Also, for calves fed the MR with 26% CP and added Lys and Met had 8% greater ADG than the calves fed the 28% CP MR without added no Lys and Met.

To obtain optimum growth (0.484 kg/d), should feed 0.68 kg/d of whey-based milk replacers at 26% CP and 17% fat, supplemented with 2.34% Lys, and 0.72% Met. This formulation gives optimum feed efficiency, and serum concentrations of urea nitrogen, while feeding calves more CP and essential AA did not improved ADG and efficiency (Hill, 2008).

Another study showed that, in 20%, CP MR fed at 0.45 kg/d, supplementing with Lys and Met improved ADG (Hill et *al*., 2007b). However, supplementing MR with AA had more effect when CP is of vegetable nature, for example; supplementing Lys, Met, Thr, and Ile has increased ADG in calves fed MR containing milk and soy proteins (Jenkins and Emmons, 1983; Kanjanapruthipong, 1998).

Objectives

3. Objectives

Determination of limiting amino acids for the growth of breastfed calves and their functionality

The amino acid requirements in suckling calves for growth have been poorly evaluated and mainly focused on essential amino acids. Recently, amino acids are taking more important role from the point of view of their functionality (such as metabolism regulators, immune system stimulators, protein synthesis stimulators, epigenetic regulators, neurotransmitters among others) regardless of whether they are essential or non-essential. This project aims to identify candidate amino acids (essential or not) that may limit the growth of milk fed calves, and to evaluate the possible functionality of some of them in order to improve their growth efficiency, muscle development and health status throughout the first 2 months of life. The use of techniques such as proteomics or computerized tomography may help to understand the changes that amino acids cause in the metabolism and body composition of calves and their mechanisms of action in improving feed efficiency.

The **hypothesis** of this project is that the formulation of milk replacers (MR) supplemented with specific amino acids will improve the efficiency of nitrogen utilization during the first 8 weeks of life of calves.

General objective

This project will generate some of the knowledge necessary to give a new approach to the formulation of MR for milk fed calves, improving the efficiency of their use and evaluating the functionality of certain amino acids throughout the first 8 weeks of life. To carry out this approach it is necessary to create a multidisciplinary team of two different institutions (the conjunction of animal production with biochemistry), in order to evaluate animal performance and the mechanisms of action involved in the process.

<u>This project is divided into 3 Work Packages</u>

1. Validation of the computed tomography (CT) technique as an estimator of the body composition of calves
2. Determination of amino acids that may be limiting for the growth of calves
3. Evaluation of the functionality of certain amino acids in milk-fed calves.

My work is based on Work Package number two.

The main objective of this thesis of Master of Science in Animal Nutrition was to study the effect of MR supplementation with amino acids on calf growth. The specific aim was to compare growth performance between calves fed a MR without amino acids and other MR supplemented with specific amino acids during 8 weeks of calves age.

Materials and Methods

4. Materials and Methods

The work has been conducted at the farm of IRTA (Institute of Food and Agricultural Research), located in Torre Marimon (Caldes de Montbui, Barcelona, España).

4.1. Animals

Seventy-one Holstein calves (seventy males and one female), and nine crossbred calves (six females and three males) were selected for the experiment. The average age at entry was (2.8 ± 1.75) days old and an average body weight of (43.5 ± 6.36 kg).

All calves came from a single private farm (Murucuc, Vic, Barcelona), located 60 km from the IRTA facilities.

Calves were housed at the IRTA barn, which is a covered housing with natural ventilation (Figure 5). In the nursery phase, calves were housed in individually adjacent pens (1×1.55 m) bedded with sawdust that was changed once a week. Calves had an *ad libitum* access to clean, fresh water.

Figure 5. Schematic representation of the IRTA barn.

Calves stayed in individual pens until 63 days of life. Then, calves were moved to collective boxes of (7×7.75 m), where they stayed in a group of five calves (Figure 6).

Figure 6. Calves in individual and collective boxes.

Calves were randomly distributed in 4 different treatments with 19 animals in each group, taking into account the homogeneity of the groups in relation to weight, sex and breed. Treatments were:

- Diet control **(MR)** was based on a milk replacer (Nukamel, The Netherlands's Company, Amsterdam, Netherland) which contains 24.5% CP and 21% fat.
- Treatment **LMT**: MR + 0.62% L-Lys+ 0.22% L-Met+ 0.61% Thr (based on Hill et al., 2007)
- Treatment **PT**: MR + 0.2% Phe + 0.2% Tyr (based on Terré et al., 2006)
- Treatment **PG**: MR + 0.3% Pro + 0.1% Gly

In addition, a limited amount of starter according to days of age was distributed, to reduce animal variability in feed intake and interference with starter feed. Ingredients content of different MR and starter are described in Table 9 and 10 respectively.

Calve’s health was monitored daily. Body temperature was recorded if calves were sick. Also, an antibiotic treatment was given to calves subjected to biopsy during the 7th week of rearing for data collection of work package 3. Fecal scores were recorded daily in a 1 to 3 scale (**1** = firm, **2** = runny, **3** = liquid).

Table 9. Ingredients content of different milk replacers used.

Constituent	Treatments			
	MR	LMT	PT	PG
WPC[1] (35%CP)	15.0	15.0	15.7	15.6
SMP[2]	39	40	40	40
Fatted whey (50% fat)	39	39	39	39
WPC 60	4.0	2.6	2.1	1.0
Premix[3]	3	3	3	3
Proline	-	0.3	-	-
Glycine	-	0.1	-	-
Phenylalanine	-	-	0.2	-
Tyrosine	-	-	0.2	-
Lysine	-	-	-	0.62
Methionine	-	-	-	0.22
Threonine	-	-	-	0.61

[1:]WPC:Whey proteins concentrate; [2:] SMP:skim milk powder; [3:]Premix: (CF : 0 %, Ca :0.8%, PH :0.7%, SO :0.5%, E321, BHT:25mg, Vit A: 25000IU, Vit D3:4500IU, Vit E and C:300mg, Iron:150mg, Ca iodate:1.3mg, Copper:10mg, Manganes:40mg, Zinc:170mg, Selenium : 0.4mg).

4.2. Feeding

The feeding of calves from the 3rd day of age to weaning at 56 days consisted mainly on: milk replacer, limited amount of starter and straw. Then, from days 54 to 63, calves received only starter and straw *ad libitum*.

4.2.1. Milk replacer

Calves were kept under their treatment (after having drunk the colostrum in the original farm) from 3 to 4 days of age up to 8 weeks of life. The MR feeding was divided into 2 equal meals at 7:00 am and 3:00 pm and dispensed with 3 liter bottles, as follows: from the 1st to 4th day, calves receive 0.5 kg DM of MR at 37ºC**.** This quantity gradually increased with time, then diminished again to 0.45 kg MR/d to facilitate weaning (Table 11).

Table 10. Ingredients and chemical composition of the starter and straw used.

Item	Starter	Straw
Ingredient % DM		
Barley	14.5	
Corn	26.3	
Wheat	13.2	
Soybean meal- 47	18.8	
Soybean husk	0.92	
Wheat middlings	11.5	
Carbonat càlcic	1.05	
Bicolucent phosphate	0.61	
Mg oxide	0.043	
Salt	0.70	
Premix[1]	0.17	
Chemical composition % of DM		
CP	14.8	3.18
Ether extract	2.28	-
ADF	4.91	44.3
NDF	13.0	66.8
Crude ash	4.38	5.36

[1:] Premix: (Ca: 0.92 %, P: 0.59%, Mg: 0.32%, K: 1.29%, S: 0.36%, Vit A: 10.150 UI, Vit D: 2.540 UI

There were two ways of preparing the milk replacer; 1) mixing manually using a scale if the quantity was small, 2) using an automatic milk tank if there was a large amount of milk. Calves were weaned at day 56 of the study and measurements were taken until day 63.

4.2.2. Starter and straw

After the morning milk distribution, the previous day's refusal of starter and straw were weighed and replaced with fresh feed according to the age of the animal. From the 1st to the 49th day, feed was offered restricted to avoid any possible effect of starter consumption on treatments. From the 50th day, onwards, calves ate starter *ad libitum.* The amount of starter offered is shown in Table 12.

Table 11. Calves liquid feeding program.

Days	Milk distributed (l)		Milk powder DM (g/d)	Milk concentration (g MR/l)
	Morning	Afternoon		
1-4	2	2	500	125
5-7	2	3	625	125
8-14	2	3	750	150
15-49	3	3	900	150
50-56	3	0	450	150

Table 12. Calves starter feeding program.

Days	Starter offered (g)	Days	Starter offered (g)
1-17	100	32-33	600
18-21	200	34	700
22-24	300	35	800
25-28	400	36-38	1000
29-31	500	39-49	1200

Table 13. Amino acids profile of MR and starter.

	MR	LMT	PT	PG	Starter
Total amino acids % (p.p) s.s.n.[1]	24.3	24.0	24.3	24.7	16.1
Aspartic acid	2.17	2.07	2.14	2.10	1.55
Glutamic acid	4.89	4.93	4.87	4.82	3.33
Serine	1.32	1.25	1.29	1.29	0.85
Histidine	0.53	0.52	0.55	0.52	0.41
Glycine	0.53	0.62	0.50	0.51	0.76
Threonine	1.37	1.30	1.36	1.75	0.70
Arginine	0.74	0.76	0.77	0.76	1.17
Alanine	0.93	0.87	0.90	0.91	0.79
Tyrosine	0.87	0.86	1.02	0.90	0.58
Valine	1.49	1.47	1.48	1.44	0.77
Methionine	0.53	0.49	0.50	0.67	0.21
Phenylalanine	0.96	0.95	1.20	0.97	0.82
Isoleucine	1.32	1.30	1.32	1.28	0.66
Leucine	2.45	2.35	2.38	2.36	1.35
Lysine	2.22	1.92	1.98	2.49	1.06
Hydroxyproline	<0.030	<0.030	<0.030	<0.030	0.043
Proline	2.00	2.32	2.03	1.92	1.05
Total tryptophan	0.33	0.33	0.34	0.36	0.19
Moisture & volatile matter (103ºC)	3.73	3.68	3.58	3.63	12.45
Crude protein (N × 6.25)	24.00	23.51	24.43	24.80	17.85

[1]: % (p.p) s.s.n: Expression of results on natural substance.

4.3. Animal measurements

Calves were weighed at the entrance to the farm, and then each week until weaning, on Tuesdays at noonday when most feed had already been digested to reduce its influence on body weight. The withers height of calves was also measured each week until weaning. On Tuesdays, after weighing with an electronic meter (Figure 7) with a measuring range of 0.6 to 16 m and an accuracy of ± 1%.

Figure 7. 16M Ultrasonic Distance Meter.

Blood samples were taken at 2, 5 and 7 weeks of life, from the jugular vein into 10 ml BD, vacutainer tubes with 170 IU of sodium heparin (BD, Belliver Industrial Estate, and Plymouth, UK), 4 hours after the morning feeding.

4.4. Indexes evaluated

The quantity of milk, starter and straw offered to individual calves according to Table 11 and 12, and the refusal, if any, where recorded to calculate the individual average of dry matter intake (aDMI)

$$aDMI = offered - refusal$$

Moreover, total DMI (tDMI) was calculated according to the expression:

$$tDMI = \sum aDMI$$

Average daily gain (ADG)

$$\mathbf{ADG} = \frac{\mathbf{Finish\ weight - Start\ weight}}{\mathbf{Age(days)}}$$

Conversion index (CI)

$$\mathbf{CI} = \frac{\mathbf{(Milk + Starter + Straw)Kg}}{\mathbf{\Delta^{o}Weight(Kg)}}$$

Efficiency

$$\mathbf{ef} = \frac{\mathbf{1}}{\mathbf{CI}}$$

4.5. Analytics

- During the three experimental months, each day, before starting work, all weighing equipment must be calibrated and adjusted.
- All analyzes of total amino acid composition of MR and starter were determined through the Office S.L. (Cereal research laboratory) which covered by ENAC accreditation following PNT-MF-109 (HPLC-Fluorescence) method,
- PNT-M-148 (HPLC-UV (DAD) method, for total tryptophan,humidity and volatile Matter (103ºC) by PNT-MF-142 (Gravimetry, Regulation (EC) No 152/2009) method.
- Finally,crude Protein (N x 6.25) was analyzed by PNT-MF-037 (Kjeldahl distillation, Regulation (EC) No 152/2009) method.
- The determination of the amino acid profile in plasma is adapted from the method described by Teerlink et al. (1994). It will be determined in76 animals at week.

4.6. Statistical analyses

Growth (BW, WH and BW:WH ratio) and intake (milk, starter and straw) data from 1 to 49, 1 to 56, 1 to 63, 50 to 56 and 56 to 63 days were analyzed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). The statistical model included terms for fixed effects of IBW or IWH used as a covariate and treatment. Calf was included as a random effect. The linear model was:

$$Y_{ijkl} = \mu + T_i + \gamma X_{ij} + C_k + \varepsilon_{ijkl}$$

Where:

Y_{ij} = response variable,

μ = overall mean,

T_i = treatment fixed effect ($_i$ = from 1 to 4),

γX_{ij} = Covariance coefficient,

C = calves random effect (n=76),

ε_{ijk} = residual error term

The least square means (LSMEANS) procedure was used to calculate mean values. When the treatment effect was significant, LSMEANS were compared using the Tukey-Kramer adjustment. The effects were considered significant at $P < 0.05$ and trends were declared at $0.05 \leq P < 0.10$.

Results and Discussion

5. Results and Discussion

Four male Holstein calves; tow from the MR treatment, one from the PG treatment and one from the LMT treatment died during the experiment for reasons unrelated to feeding treatments. Therefore, the study refers to data from 67 Holstein and 9 crossbred calves (19 animals per treatment), with an initial age of 2.7 ± 1.77 days, and an initial BW of 43.5 ± 6.39 kg.

Target nutrient concentrations of the various MR powders were achieved. All MR offered was consumed. No statistical analysis was made for fecal scores, because the incidence of diahrrees was low.

We divided the rearing period which lasts approximately 2 months (63 days) in different phases, to better control the consumption of different feeds (milk, concentrate and straw), as following:

- From day 1 to 49 of the experiment: calves received amounts of milk which increase gradually starting from 500 until 900 g/d and limited amount of starter(an average of 310 g/d) and straw (an average of 10g/d).
- From day 1 to 56 of the experiment: This phase corresponds to the lactation period until weaning at 56 days.
- From day 1 to 63 of the experiment: This phase encompasses the entire rearing period.
- From day 50 to 56 of the experiment: Reduction in the amount of milk dispensed until 450 g/d and *ad libitum* distribution of starter and straw.
- From day 56 to 63 of the experiment: Distribution of only the starter and straw *ad libitum.* This phase corresponds to the post weaning.

5.1. Feed intake

There were no significant differences (P > 0.05) between treatments MR, PG, PT and LMT concerning the consumption of DM of milk, starter and straw, during the pre weaning phase and one week post weaning (Table 14).

Table 14. Average daily and total milk, starter, and straw intake by Holstein calves fed different milk replacer (MR) and starter programs.

Item[d]	Treatments[a]				SEM[b]	P-Value[c]
	MR	PG	PT	LMT		
Milk (kg of DMI)						
1-49d	41.3	41.2	41.4	41.3	0.14	0.76
1-56d	44.5	44.3	44.6	44.4	0.14	0.63
1-63d	44.5	44.3	44.6	44.4	0.14	0.63
50-56d	3.15	3.10	3.15	3.11	0.026	0.41
Starter (kg of DMI)						
1-49d	15.9	15.4	14.8	14.5	0.92	0.72
1-56d	25.7	25.0	24.4	23.8	1.30	0.75
1-63d	41.9	40.0	39.6	39.2	1.84	0.73
50-56d	9.84	9.53	9.56	9.31	0.548	0.92
56-63d	17.9	16.6	16.8	17	0.79	0.63
Straw (kg of DMI)						
1-49d	1.43	1.63	1.56	1.64	0.144	0.70
1-56d	2.10	2.29	2.29	2.29	0.167	0.79
1-63d	2.89	2.39	3.15	3.04	0.184	0.02
50-56d	0.67	0.66	0.72	0.65	0.044	0.62
56-63d	0.91	0.87	0.99	0.84	0.058	0.30
SDMI (kg of DMI)						
1-49d	17.3	17.1	16.4	16.2	0.97	0.81
1-56d	27.8	27.3	26.7	26.1	1.36	0.82
1-63d	44.8	42.4	42.7	42.2	1.90	0.75
50-56d	10.5	10.2	10.3	10.0	0.56	0.91
56-63d	18.8	17.4	17.8	17.8	0.80	0.63
TDMI (kg of DMI)						
1-49d	58.6	58.3	57.8	57.5	1.03	0.86
1-56d	62.4	62.0	61.7	61.2	1.03	0.85
1-63d	89.2	86.7	87.3	86.6	1.95	0.75
50-56d	13.6	13.3	13.4	13.1	0.56	0.89
56-63d	19.3	17.8	18.2	18.3	0.80	0.61

[a]: MR: milk replacer with 24.5% CP and 21% fat, as control, PG: MR + Proline +Glycine, PT: MR + Phenylalanine +Tyrosine, LMT: MR+ Lysine + Methionine + Threonie.

[b]S.E.M. =standard error of the mean

[C]:effect of feeding treatments

[d] DMI: dry mater intake, SDMI: solid dry mater intake=(Starter DM+Straw DM), TDMI: total dry mater Intake
1-49: solid intake restricted, 1-56: weaning, 1-63:total breeding period, 50-56: *ad libitum* solid intake with MR, 56-63: just *adlibitum* solid intake.

The evolution of milk intake throughout the study was no significant (P = 0.63) from the first day until weaning at day 56. The milk DM intake was the same (P = 0.76) for different treatments, with an average of 41.3 kg of DMI during the first 49 days, and an average of 44.5 from the first day of rearing to weaning at day 56 (Figure 8).

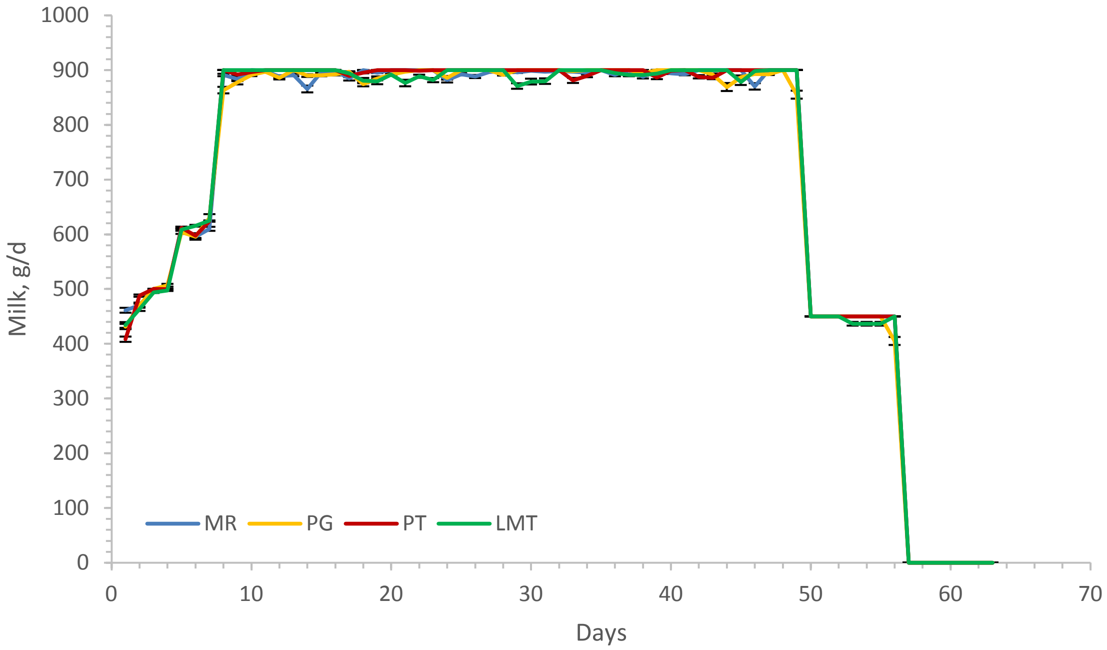

Figure 8. Average daily DM milk consumption in different treatments.

The total amount of starter consumed was the same for calves receiving MR without AA compared with calves receiving MR with AA during the entire period of rearing. No difference (P > 0.05) in starter and straw feed intake was observed between calves offered MR or PG, PT and LMT treatments (Table 14).

According to the Figure 9 and 10, calves during the first 15th days did not consume starter and straw which is normal. Because, the rumen of a newborn calf is undeveloped physically, and nonfunctional metabolically (Warner et al., 1956). The initiation of solid feed intake can helps the rumen in his physical and metabolic development.

Graphs 9 and 10 have the same appearance but are different from that of milk. There is an inverse relationship between milk intake and starter intake (Terré et *al*., 2007; Raeth-Knight et al., 2009). There was an increase in starter and straw consumption for different treatments one week before weaning when the amount of milk dispensed was reduced to 450 g/d and this increase persists even after weaning. Several commercial programs that promote high feeding rates of high protein MR instruct feeders to wean calves gradually over 7d by feeding half of the MR in a morning-only feeding regimen (Hill, 2010), as it has been done in our experiment to overcome the slump in performance. Our results are in agreement with those of Khan et al., (2007 a, b), who observed greater starter and straw intake when milk-feeding levels were restricted, typically equivalent to 10% of BW of milk offered.

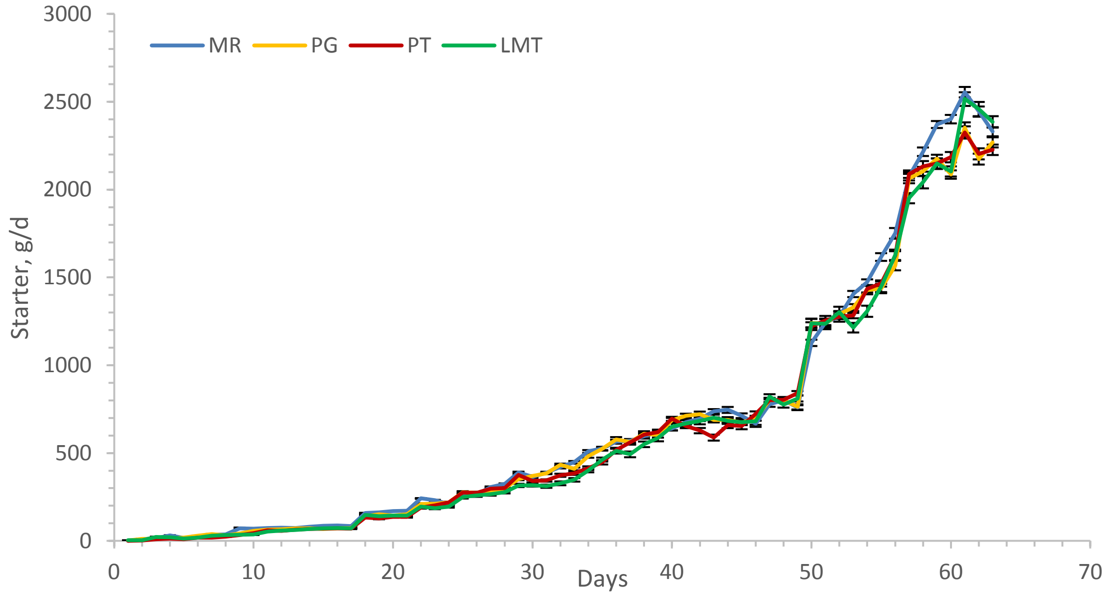

Figure 9. Average daily DM starter consumption in different treatments.

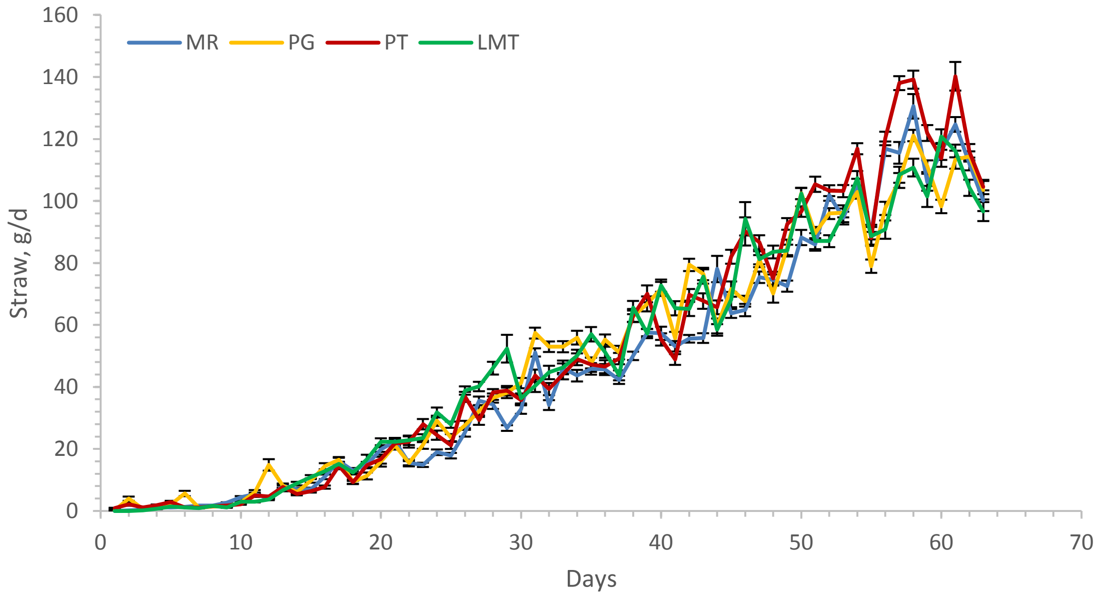

Figure 10. Average daily straw DM consumption in different treatments.

Total dry matter intake

There were no differences in total DMI (Milk, Starter and Straw) between calves among different treatments (*P* > 0.05) in different phases of rearing (Table 14). From day 1 to 63 there was no difference (*P* = 0.75), but numerically, calves on MR treatment consumed 1.9 kg of total DM more than calves on PT and 2.5 kg of total DM more than calves on PG and LMT treatments. Calves eat an average of 87.5 kg of total DM during 63 days of rearing (Figure 11) which corresponds to accelerated feeding program.

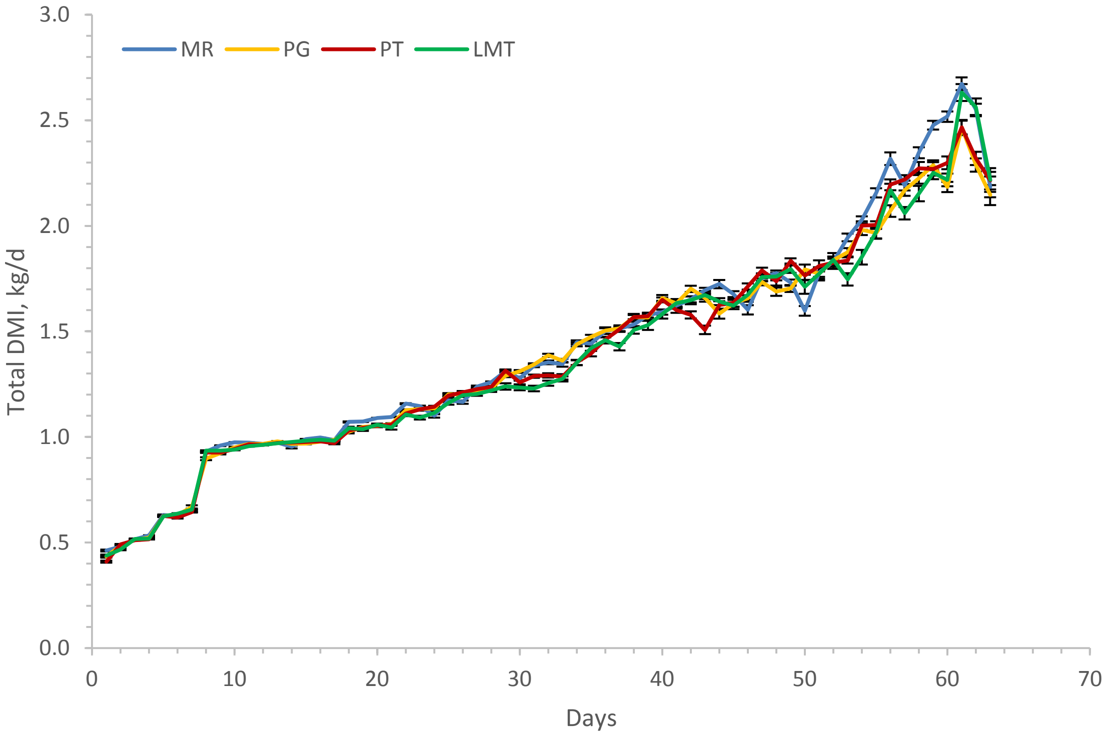

Figure 11. Total dry matter intake for different treatments.

5.2. Growth performance

5.2.1. Body weight and wither height

In this experiment, there were no differences (*P* > 0.05) in the initial measurements of body weight (BW), wither height (WH) and BW: WH ratio among treatments (Table 15). We recorded an average body weight of 88.9 kg at weaning (56d) and 95.0 kg as final body weight at day 63. In addition, there were no differences in WH between treatments (*P* > 0.05) with an average of 98 cm. However, numerically, MR treatment had the highest value of WH (100 cm) followed by PG treatment (99 cm). The ratio of BW to WH increases linearly with age (Kertz et al., 1998) (Table 15).

Table 15. Performance parameters of Holstein calves fed different milk replacer (MR) and starter programs.

Item[d]	Treatments[a]				SEM[b]	*P*-Value[c]
	MR	PG	PT	LMT		
Initial						
BW (kg)	44.2	44.3	42.5	42.5	1.52	0.72
HW(m)	0.84	0.84	0.82	0.83	0.008	0.49
BW:HW	52.8	52.9	51.7	51.4	1.50	0.61
at 49d						
BW (kg)	83.9	85.5	82.1	81.5	1.81	0.40
HW(m)	0.98	0.98	0.97	0.93	0.021	0.23
BW:HW	85.6	87.6	84.9	88.1	3.69	0.75
at 56d						
BW (kg)	90.4	90.0	87.5	87.6	2.16	0.66
HW(m)	1.00	0.99	0.98	0.95	0.009	0.76
BW:HW	90.7	90.9	89.3	92.3	1.84	0.45
at 63d						
BW (kg)	98.9	94.5	92.9	93.8	2.43	0.29
HW(m)	1.10	0.99	0.99	0.97	0.066	0.57
BW:HW	89.9	95.4	93.9	96.7	2.52	0.62

[a]: MR: milk replacer with 24.5% CP and 21% fat, as control, PG: MR + Proline +Glycine, PT: MR + Phenylalanine +Tyrosine,

LMT: MR+ Lysine + Methionine + Threonie.

[b]S.E.M. =standard error of the mean

[C]:effect of feeding treatments

[d]: BW: body weight, HW: high at weither, BW: HW : ratio body weight : high at weither.

5.2.2. Growth indicators

No difference noted ($P > 0.05$) between calves fed MR and other treatments supplemented with AA concerning average daily weight gain. For example, from one to 56 days, the average weight gain was 787.3g/d and from day 1 to 63 was 835.5 g/d. We also, observed the same feed efficiency for different treatments ($P > 0.05$). From day 1 to day 56, the feed efficiency was 0.64 and from day 1 to 63 was 0.61 (Table 16).

Table 16. Performance of calves fed different milk replacer (MR) and starter programs.

ITEM[d]	Treatments[a]				SEM[b]	*P*-Value[c]
	MR	PG	PT	LMT		
1-49						
ADG (g/d)	814	814	797	808	18.9	0.92
CI	1.47	1.51	1.50	1.46	0.028	0.58
Efficiency	0.68	0.67	0.67	0.69	0.013	0.53
1-56d						
ADG (g/d)	805	784	786	774	23.5	0.81
CI	1.55	1.60	1.59	1.55	0.032	0.53
Efficiency	0.65	0.63	0.63	0.65	0.012	0.50
1-63d						
ADG (g/d)	865	815	823	839	27.6	0.56
CI	1.64	1.69	1.69	1.64	0.037	0.65
Efficiency	0.61	0.60	0.60	0.61	0.013	0.59
50-56d						
ADG (g/d)	1016	938	961	874	77.8	0.61
CI	1.99	2.22	2.71	2.32	0.403	0.63
Efficiency	0.52	0.47	0.49	0.47	0.031	0.66
56-63d						
ADG (g/d)	1253	949	897	1066	98.4	0.06
CI	2.33	2.89	3.95	2.85	0.545	0.21
Efficiency	0.45	0.38	0.33	0.41	0.030	0.04

[a]: MR: milk replacer with 24.5% CP and 21% fat, as control, PG: MR + Proline +Glycine, PT: MR + Phenylalanine +Tyrosine,

LMT: MR+ Lysine + Methionine + Threonie.

[b]SEM =standard error of the mean

[C]:effect of feeding treatments

[d] ADG: average daily gain, CI : conversion indice, 1-49: solid intake restricted, 1-56: weaning, 1-63:total breeding period, 50-56: *ad libitum* solid intake with MR, 56-63: just *adlibitum* solid intake.

The addition of the AA did not give high values compared to MR without AA concerning ADG and feed efficiency (Figure 12 and 13) that may be due to the high content of CP in MR (24.5%). This makes energy limiting for the use of supplemental AA, or may be explained by the minimal dose of AA (0.62% of Lys, 0.22% of Met and 0.61% of Thr) which did not give effect or was not sufficient to stimulate growth in calves. Because, Hill et *al*. (2008), observed 17% improvement in ADG with feeding MR 24% CP and 17% fat supplemented with 2.34 Lys, 0.72 Met, but no effect was noted with adding Thr.

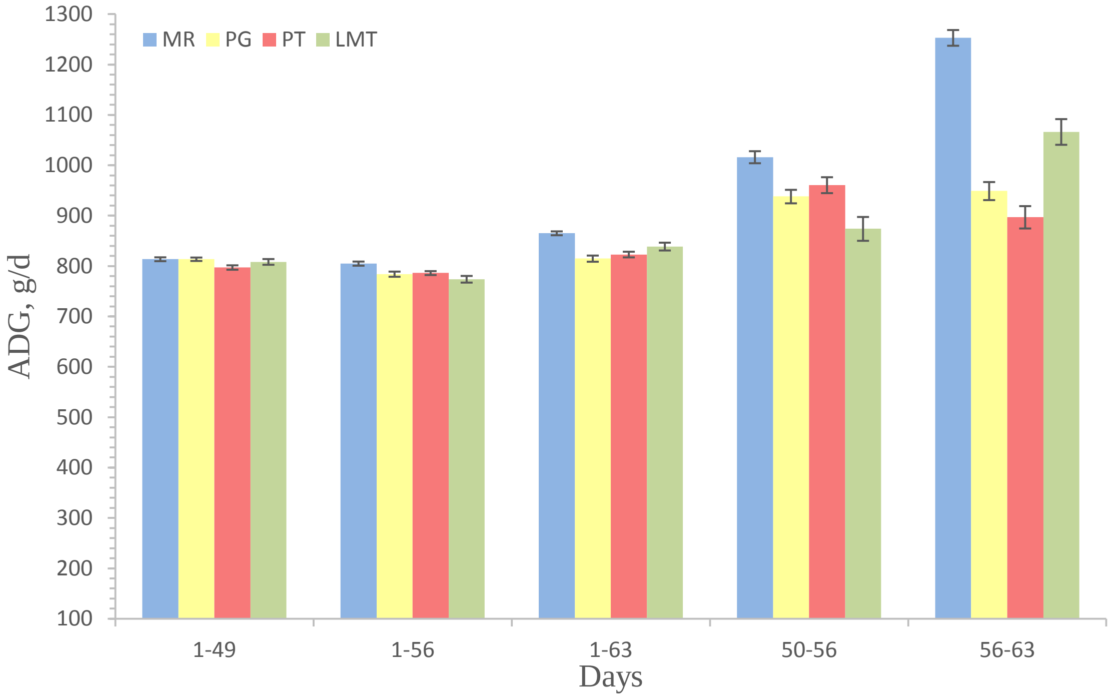

Figure 12. Average daily weight gain of different treatments for calves in lactation.

Another hypothesis which can explain our results; a study of Hill et *al.* (2009a, b) that reported that ADG was less in calves fed high fat MR (20, 23, and 27% fat in the MR powder) than in calves fed MR with 17% fat, and differences in starter intake did not explain the difference in ADG, in our case fat was equal to 21%.

Because the nutrients digestion decrease as fat concentration increased from 13 to 23% of a 27% CP MR Powder fed at 0.66 kg of DM/d which explained much of the differences in ADG (Hill et *al.*, 2009b).

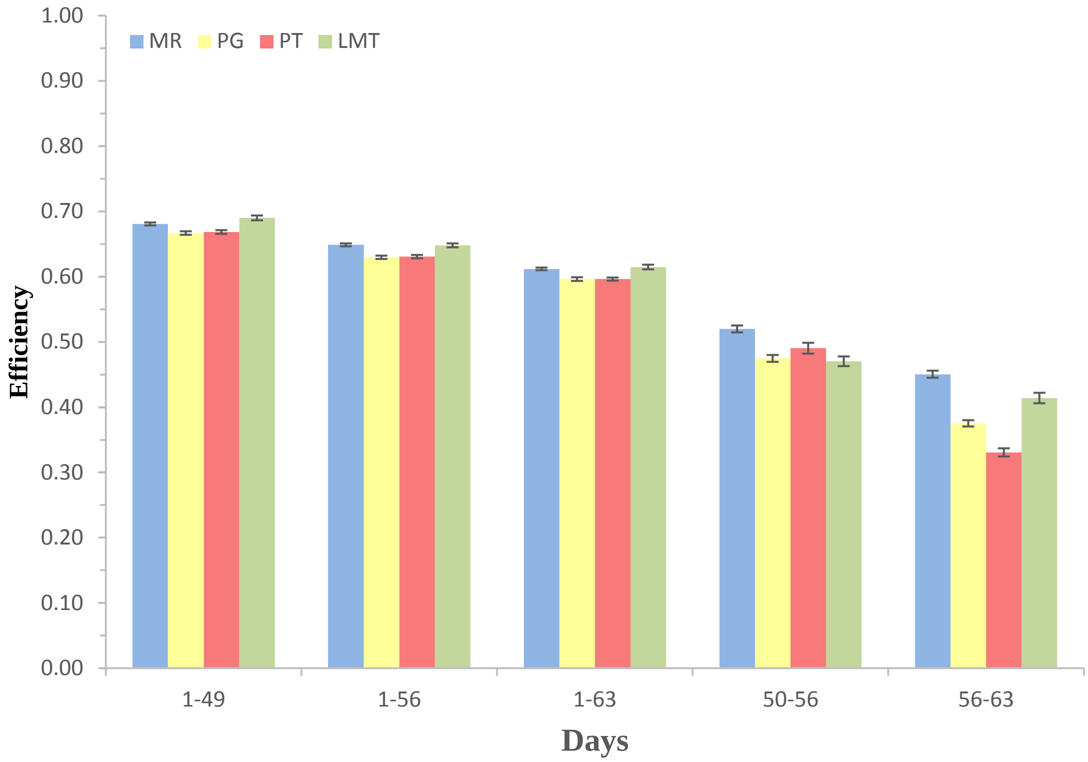

Figure 13. Efficiency of different treatments for calves in lactation.

5.3. Discussion

This no significant difference in this study concerning consumption, weight, height and average daily gain can be explained by several hypotheses:

There was no significant difference in TDMI between different treatments with or without AA, which means that the animal body did not benefit from this supplementation. This can be explained by the energy to protein ratio (0.86) in the MR, in our case with accelerated growth either energy may be limiting growth or AA supply was in excess, or both. Terre (2006) noted the same results for no utilization of AA in enhanced feed calves and reported that the energy to protein ratio was too low to promote an adequate utilization of AA. Thus, two solutions can be envisaged, either decreases the level of CP or increases the content of fat in the MR to improve the use of CP.

There was also no difference in calf growth, with supplementation of amino acids, which may be due to the insufficient dose of the amino acids added to the MR, which was 0.22% Met, 0.62% Lys and 0.61% Thr. These doses were lower and did not give any effect by comparing them with those of Hill (2008), he used 0.72% Meth which is more than 3.3 times the dose used in our study, 2.34% Lys which is more than 3.8 times compared with our and 1.34% Thr which is more than 2.2 times. There were no differences *(P* > 0.05) for ADG, starter intake, or efficiency relative to Thr concentration of the MR, no response concerning the addition of threonine was observed.

Another hypothesis which can explain our results is the nature of CP added to MR. Supplementing MR with AA had more effect when CP was of vegetable nature, for example supplementing Lys, Met, Thr, and Ile increased ADG in calves fed MR containing milk and soy proteins (Jenkins and Emmons, 1983; Kanjanapruthipong, 1998), and dosis of CP, Hill (2008) reported in his experiment that when feeding calves MR with 28% CP and supplemented with Lys and Met; he didn't observe an increase in ADG and efficiency compared with calves fed MR with 28% CP without added Lys and Met. Thus, the efficiency of utilization of absorbed amino acids (AA) could be improved by lowering the CP content of MR, and adjusting its amino AA profile (Terre, 2006). However, there was significant effect *(P* < 0.05) for calves fed MR at lower CP concentrations with added AA and noted greater ADG and efficiency than calves fed the MR without added Lys and Met.

Also, it could be that NEAA such as Proline and Glycine are not really needed in the case of metabolic effects.

Conclusion

6. Conclusion

The results of the present study show that feeding MR contained high protein and fat (24.5% CP, 21% Fat) and supplemented with specific AA (Lys Meth Thr, Ph Tyr or Gly Pro), compared with the same MR without AA in calves of 8 weeks of age, didn't improve in calf's performance. No differences were detected in feed intake or growth performances during all the experiment. Our results from day 1 to weaning at day 56 were an average of 61.8 kg of total dry matter intake, an average daily gain of 787.3 g/d and a feed efficiency equal to 0.64. At weaning, we noted 88.9kg, 98 cm and 0.91 respectively for an average BW, WH and BW: WH ratio.

In conclusion, added AA treatments during the pre weaning period at day 56 had no effect. No differences in ADG and feed efficiency were found between different treatments.

Therefore, feeding calves with an MR that contains 24.5% CP and 17% fat contains sufficient amino acids to support the target growth.

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