Evaluation of milk replacer supplemented with lysine and methionine in combination with glutamate and glutamine in dairy calves

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ABSTRACT

This study aimed to evaluate performance, gut health and metabolic changes in dairy calves receiving milk replacer supplemented with lysine and methionine with 0.6% or 1% AminoGut (10% Glutamate and 10% Glutamine). Forty-five Holstein calves were blocked and distributed in the treatments: (1) Control: no supplementation; (2) Lys, Met, AminoGut 0.6%; (3) Lys, Met, AminoGut 1%. Animals were individually housed, with free access to water and concentrate. Milk replacer (6 L/d) was fed until the eighth week of life. Feed intake and faecal score were monitored daily. Calves were weighed and measured weekly. Blood samples were collected every other week to determine metabolites. Supplementation did not affect the performance nor the metabolism of calves. There was no effect of supplementation for faecal score. An age effect was observed for most of the variables, mainly as a response of starter intake and consequent shift from a pre to a functional ruminant metabolism. The supplementation of the milk replacer with lysine and methionine in association with 0.6% or 1% AminoGut had no effect on performance, gut health nor metabolism of dairy calves. More important than the fine adjustment of amino acid requirements is the adoption of practices to improve starter intake.

Introduction

One of the biggest challenges in calf rearing is cost reduction without affecting performance. The substitution of whole milk by milk replacer and the inclusion of products that stimulate starter intake, to guarantee early weaning, are excellent alternatives (Noori et al. 2016; Huuskonen 2017).

The use of non-milk protein in the formulation of milk replacers in addition to the lack of definition of calves’ essential amino acids requirements are some of the reasons that lead to a failure of these products to meet the amino acid requirements of pre-ruminant calf. However, the recommendation of Hill et al. (2008) of 17 g/d lysine and 5.3 g/d methionine has been shown to meet the requirements of calves less than 5 weeks of age. Lysine (Lys) and methionine (Met) are the main limiting amino acids for growth (Williams and Hewitt 1979; Tzeng and Davis 1980; Hill et al. 2008; Li et al. 2008). According to Silva (2014), all of the milk replacers sold in Brazil do not supply Lys or Met for calves to achieve their requirements, regardless of volume fed, which may lead to reduced growth.

Another factor that reduces growth rate is the occurrence of diarrhoea, which also is the main cause of mortality and morbidity. Glutamate (Glu) and glutamine (Gln) are widely used in monogastric nutrition, and besides stimulating consumption, reduce intestinal disorders such as diarrhoea. Glutamine restores intestinal mucosa and reduces the detrimental impact caused by diarrhoea (Reeds et al. 2000). Glutamate, the main constituent of the dietary protein (Burrin and Stoll 2009), is the most abundant intracellular amino acid, and can be used to form other amino acids responsible for the maintenance and integrity of the intestinal mucosa. In pigs, the addition of glutamate in the diet has been linked to increased feed intake and consequently increase body growth rate (Gatel and Guion 1990). Thus, it is possible that supplementation of calves with glutamate and glutamine, besides stimulating starter intake and assisting in intestinal health, may improve the metabolism of nitrogen compounds. Glutamate plays a key role in catabolism in the removal of α-amino nitrogen. Supplementation with this amino acid, along with other limiting amino acids, may have additive effects on protein metabolism, resulting in higher growth rates.

The aim of this study was to evaluate the effect of supplementation of the milk replacer with lysine and methionine to achieve requirements and its association with glutamate and glutamine on performance, intestinal health and metabolism of dairy calves.

Material and methods

All experimental procedures were approved by the Animal Care and Use Committee of University of São Paulo and were performed in accordance with their guidelines.

Forty-five Holstein male calves from a commercial farm (2 ± 2 d of age) were blocked considering body weight of the animals when they reached the experimental area. After birth, calves...
received 2 L of high-quality colostrum during the first two meals. Calves were transported to the experimental calf facility at the Agriculture College, University of Sao Paulo, and housed in individual hutches with free access to water and starter concentrate during the entire experimental period. All calves were fed with 6 L/d of milk replacer (Violet Spray®, Sao Paulo, Brazil), crude protein: 21.17%, ether extract: 15.58%, total solids: 12.5% divided into two meals (07 and 17 h), until the eighth week of life when calves were abruptly weaned. Calves were randomly distributed to one of the following treatments: (1) Control: commercial milk replacer without amino acids supplementation; (2) Lys, Met, AminoGut 0.6%: milk replacer supplemented with Lys and Met plus 0.6% AminoGut (10% Glutamate and 10% Glutamine), resulting in an intake of 17, 5.3, 0.43 and 0.43 g/d of Lys, Met, Glu and Gln, respectively; and (3) Lys, Met, AminoGut 1%: milk replacer supplemented with Lys and Met plus 1% of AminoGut, resulting in an intake of 17, 5.3, 0.672 and 0.672 g/d of Lys, Met, Glu and Gln, respectively. Lys and Met were supplemented to reach consumption of 17 and 5.3 g/d, respectively, maintaining a Lys:Met ratio of 3.2:1. Milk replacer supplementation was performed by adding to each 25 kg of milk replacer 249.6 g L-lysine (320 g L-lysine Monohydrachloride 99%, Ajinomoto do Brasil Ind. e Com. de Alimentos Ltda), 108.9 g L-methionina (110 g Rhodomet, Adisseo) and 143 g AminoGut for the treatment AminoGut 0.6% or 240 g AminoGut for the treatment AminoGut 1%. For determination of amino acid profile present in the commercial milk replacer, the NIRS calibration curve for dairy products validated by Adisseo Brasil Nutrição Animal Ltda. was used. Samples of starter concentrate and milk replacer were collected during the experimental period for subsequent analysis (Table 1).

Animals were weekly weighed in a mechanical scale before the morning feeding, until the tenth week of life, when the study ended. Faecal score was monitored daily as described by Larson et al. (1977) regarding the fluidity of faeces, being: (1) normal and firm, (2) loose but with a general healthy aspect, (3) very loose but not watery separation, (4) watery and (5) very watery. Health problems were monitored and treated according to the veterinary recommendation. All calves presented with diarrhoea at least once during the trial and were offered oral electrolytes every time faecal score was higher than 2.5.

Blood samples were collected at weeks 2, 4, 6, 8 and 10, always two hours after morning feeding, through jugular vein puncture by evacuated tubes (Vacuette of Brazil, Campinas, SP, Brazil). Samples were centrifuged (Universal 320R, Hettich, Tutlingen, Germany) at 2000g, for 20 min at 4°C, and plasma or serum was freezer stored (−26°C) until subsequent analysis. Specific commercial enzymatic kits (Labtest Diagnóstica S.A., Lagoa Santa, MG, Brazil) were used to analyse plasma glucose, alkaline phosphatase, plasma urea nitrogen (PUN), total serum protein (TSP), serum creatinine and serum albumin. β-hydroxybutyrate (BHBA) was determined using a commercial kit RANBUT (Randox Laboratories, Life Sciences Ltda., Crumlin, UK). Determinations were performed in an automatic biochemistry system (SBA-200, CELM, Barueri, SP, Brazil).

Data were analysed by the Statistical Analysis System (SAS, 1991), according to Proc Mixed, with the collection days as repeated measures. Treatments comparison was carried out according to the adjusted Tukey test, with an adopted significance level of 5%. Repeated measures were analysed according to the model: Yijk = μ + Ti + j + Tlij + Bk + eijk where: μ = overall average; Ti = Treatment Effect; j = effect of age; Tlij = Interaction Treatment × Age; Bk = Block effect; eijk = experimental error.

Results

The inclusion of amino acids increased the percentage of crude protein in the milk replacer. Percentage of ether extract in the supplemented milk replacer was lower than in milk replacer without supplementation (Table 1).

There was no effect of Lys and Met supplementation in association with glutamate and glutamine for body weight, body weight gain or growth measures (P > .05; Table 2). There was also no effect of the interaction treatment × age of the animals for this parameter (P > .05) with exception of gains of withers height and heart girth that presented interaction treatment × age (P < .05). However, there was an age effect for all the mentioned variables, since calves were in the growth phase (P < .05; Table 2).

Starter intake was not affected by amino acid supplementation (P > .05; Table 3). However, there was a significant effect of age (P < .05) and an interaction effect of supplementation and age (P < .05), with lower intake at weaning for the control calves. There was no effect of amino acid supplementation or interaction between supplementation and age of the animals on dry matter intake (P > .05). Total dry matter intake was very similar among treatments during the pre-weaning phase, even though at the sixth week animals fed with supplemented milk replacer had numerically higher dry matter intake, suggesting some supplementation effect. Most of the dry matter intake was composed by replacer intake (Table 3). However, animals quickly responded increasing dry matter intake at the first week after weaning (Figure 1). Feed efficiency during the pre- and post-weaning was not influenced by supplementation (Table 3). Likewise, there was no effect for age or interaction age × treatment for this parameter (P > .05).

Amino acid supplementation did not show a beneficial effect on the faecal score, as there was no significant effect of supplementation or the interaction age × treatment (P > .05). The incidence of diarrhoea was low during the experimental period, observed only in the first weeks of life of the animals (Figure 2).

Plasma glucose, BHBA, alkaline phosphatase, total protein, albumin and PUN were not affected by the amino acids

Table 1. Milk replacer and starter concentrate chemical composition.

| Milk replacer | Lys and Met |
|---------------|-------------|
| Control       | Aminogut    |
| Dry matter, % | 95.94       |
| Ash, % DM     | 7.84        |
| Crude protein | 21.17       |
| DM            | 15.58       |
| Gross energy  | 4360.12     |

Notes: Milk replacer, milk replacer without lysine and methionine supplementation; Milk replacer with AminoGut, milk replacer supplemented with lysine, methionine, 0.6% and 1% of Aminogut; Starter, starter concentrate.
supplementation or by the interaction of supplementation and age (P > .05). However, there was an effect of age for all of them (P < .05; Table 4; Figure 3), suggesting metabolism shift as a response of the rumen development.

Discussion

It was expected that supplementation with amino acids in the milk replacer would result in improvements in performance; however, in the present study, no differences were observed (Table 2). Literature shows that animals receiving a liquid diet supplemented with lysine and methionine presented higher body weight (Hill et al. 2007, 2008). Moreover, the addition of glutamate and glutamine in the diet is associated with an increase in the total dry matter intake and consequently improvements in the performance (Drackley et al. 2006; Hill et al. 2007; Li et al. 2008). Studies with calves supplemented with glutamate and glutamine are scarce. Nevertheless, a study with piglets receiving glutamate in the diet pre- and post-weaning, reported an increase in body weight (Gatel and Guion 1990). Likewise, a study carried out with pigs in the weaning phase reported that the addition of 1% of glutamine from soy protein in the diet increases body weight compared to animals that did not receive glutamine (Wu et al. 1996). However, no benefit in body weight was observed during the experimental period as a result of amino acids supplementation.

Weight gain in calves is a consequence of total dry matter intake, and the inclusion of glutamate and glutamine in the milk replacer aimed indirectly stimulate starter intake, due to the lower incidence of diarrhoea. However, there were no effects of amino acids supplementation on starter intake. Calves were fed 6 L/d of milk replacer, which may be responsible for the observed low starter intake. Drackley et al. (2006) in a study with calves receiving milk replacer with soy protein supplemented with L-Glutamine founded values of weight gain (282 g/d) close to those observed in the present study during the feeding phase. Regarding growth measures, withers height was similar but heart girth was smaller to that found by Drackley et al. (2006) in calves receiving glutamine in the milk replacer. Moreover, studies show improvements in growth measurements in calves fed diet supplemented with

### Table 2. Performance of dairy calves receiving the control or the milk replacer supplemented with lysine and methionine in combination with 0.6% or 1% of Aminogut.

| Treatments | Lys and Met | SEM | T | A | T×A |
|------------|-------------|-----|---|---|----|
| Control    |             |     |   |   |    |
| Aminogut 0.6% | 37.0 | 36.8 | 38.0 | 1.3 | .91 | <.05 | – |
| Aminogut 1%   | 48.2 | 48.3 | 47.2 | 1.9 | .96 | <.05 | – |
| Tenth week | 54.8 | 56.1 | 54.8 | 2.5 | .99 | <.05 | – |
| Average total period | 44.9 | 44.7 | 44.1 | 1.5 | .93 | <.05 | .09 |
| Average daily gain, kg | 246.8 | 270.6 | 218.7 | 48.3 | .76 | 0.16 | .20 |
| Pre-weaning phase | 430.7 | 548.4 | 560.2 | 79.2 | .42 | <.05 | .23 |

Notes: Control, basal diet without supplementation with amino acids; Lys, Met, AminoGut 0.6%: milk replacer supplemented to meet the requirement Lysine (17 g/d) and Methionine (5.3 g/d), and addition of 0.6% Aminogut containing 10% Glutamate and 10% Glutamine; Lys, Met, AminoGut 1%, milk replacer supplemented to meet the requirement of Lysine (17 g/d) and Methionine (5.3 g/d), and addition of 1% Aminogut containing 10% Glutamate and 10% Glutamine; SEM, Standard error of the mean; T, treatment effect; A, age effect (week); T×A, interaction Treatment × Age.

### Table 3. Starter intake, total intake and feed efficiency of dairy calves receiving the control or the milk replacer supplemented with lysine and methionine in combination with 0.6% or 1% of Aminogut.

| Treatments | Lys and Met | SEM | T | A | T×A |
|------------|-------------|-----|---|---|----|
| Control    |             |     |   |   |    |
| Aminogut 0.6% | 61 | 106.7 | 94.2 | 23.3 | .36 | <.05 | .05 |
| Aminogut 1%   | 925.5 | 1085.1 | 1008.8 | 143.1 | .73 | <.05 | .27 |
| Total intake, gDM/d | 786.5 | 843.1 | 806 | 40.6 | .61 | <.05 | .27 |
| Feed efficiency | 926.3 | 1089.3 | 1077.8 | 133.9 | .62 | <.05 | .39 |

Notes: Control, basal diet without supplementation with amino acids; Lys, Met, AminoGut 0.6%: milk replacer supplemented to meet the requirement Lysine (17 g/d) and Methionine (5.3 g/d), and addition of 0.6% Aminogut containing 10% Glutamate and 10% Glutamine; Lys, Met, AminoGut 1%, milk replacer supplemented to meet the requirement of Lysine (17 g/d) and Methionine (5.3 g/d), and addition of 1% Aminogut containing 10% Glutamate and 10% Glutamine; SEM, Standard error of the mean; T, treatment effect; A, age effect (week); T×A, interaction Treatment × Age.
Lys and Met (Hill et al. 2009, 2010). The average weekly gain for withers height is below of that found in the literature, with values close to 1.2–1.4 cm/week (Hoffman 1997; Heinrichs et al. 2003). However, data show an age effect for all performance variables because of animals’ growth.

The age effect was also observed for starter intake and total intake, both pre and after weaning (Table 3). Calves increase their solid feed intake as they grow and their energy and protein requirements increase (Figure 1). However, regardless of the treatment, starter intake was low during the pre-weaning phase and below the 700 g/d expected for calves for adequate weaning (Quigley 1996). The low starter intake may be related to the amount of milk supplied to the animals, since the higher the liquid diet intake, the lower the concentrate intake (Khan et al. 2007; Soberon et al. 2012; Bach et al. 2013). Since calves should be consuming enough concentrate for adequate weaning, alternatives for increasing intake when calves receive higher volumes of milk must be pursued (de Paula et al., 2017). However, according to the meta-analysis of Gelsinger et al. (2016), the supply of 6 L/d (about 750 g DM of milk replacer) should have not decrease the starter concentrate intake as much as it was observed.

Figure 1. Starter and total feed intake (g DM/d) of calves fed the control or the milk replacers supplemented with amino acids.

Figure 2. Faecal scores of calves fed the control or the milk replacers supplemented with amino acids.
From their data, calves fed 6 L/d should be consuming about 500 g pre-weaning. Huuskonen (2017) observed an average of 650 g/d of starter intake of calves fed 0.75 kg DM/d of three different milk replacer. Waldern and Van Dyk (1971) reported that the addition of glutamate in the concentrate increases starter intake by pigs, but decreases in calves during the milk-feeding phase. Even though literature shows that glutamate could indirectly improve intake, by improving intestine health of diarrhoeic calves, supplementation had no effect during the period of higher diarrhoea occurrence. However, even though calves were weaned without the desired starter intake to maintain growth rates, calves rapidly respond increasing starter intake, with no differences among treatments after weaning.

Starter intake affects rates of weight gain and skeletal body growth of calves in the liquid-feeding phase, and this parameter is strongly influenced by health condition of the calf. The main disturbance that affects calves in the early stages of life is diarrhoea and it normally occurs between the seventh and the tenth day of life (Von Buenau et al. 2005). In this study, it was expected that diarrhoea would be less frequent in animals receiving AminoGut supplementation, since the addition of glutamate and glutamine in the diet usually decreases the occurrence of this disorder (Reeds et al. 2000). According to Drackley et al. (2006), glutamine is an important fuel for intestinal epithelial cells, assisting in maintaining intestinal integrity when supplied in the diet for many mammalian species. Studies with monogastrics show that the incidence of diarrhoea in piglets in the post-weaning phase, a period of higher incidence in pigs, is reduced with the inclusion of AminoGut in the diet (Gatel and Guion 1990; Wu et al. 1996).

Regarding blood metabolites, there were no amino acid supplementation effects for all the evaluated metabolites. However, an age effect was observed for all the evaluated metabolites (Table 4; Figure 3), as a response of increased starter intake and the resulting rumen development and shift from a pre-ruminant to a functional ruminant metabolism. Pre-weaning plasma glucose concentration was similar to data observed for calves receiving a larger volume of liquid diet (Reece 2006). Khan et al. (2007) also observed glucose concentrations close to 90 mg/dL in calves receiving a higher volume of liquid diet. Quigley et al. (2006) observed higher concentrations of glucose for calves during the pre-weaning phase, and reduction after weaning, as it was observed in the present study. After weaning, there was a reduction in plasma glucose concentration in supplemented calves (Figure 3), which may be associated with ruminal development. This suggestion may be corroborated by the concentrations of BHBA, which were initially low, but increased rapidly after weaning. The curve shape of BHBA (Figure 3) is very similar to that of the starter intake (Figure 1), as these two variables are strongly and positively correlated (Suarez-Mena et al., 2017). A higher starter intake was expected during the pre-weaning phase, since milk replacer was supplemented with consumption-stimulating amino acids; however, calves were weaned presenting lower intake than recommended. These results suggest that although calves were not prepared for weaning, they quickly adapted to a new diet, as pointed out in several studies (Klotz and Heitmann 2006; Khan et al. 2007, 2008; de Paula et al., 2017). Decreasing glucose and increasing BHBA concentrations are indicative of ruminal development in calves and the shift from a non-functional to a functional ruminant metabolism (Lane et al. 2000; Suarez-Mena et al., 2017). However, these changes are strongly correlated with starter intake, as observed in this study. Other indicative of rumen development is the concentration of PUN. According to Eicher-Pruijett et al. (1992), increases in PUN concentration are due to an increase in solid feed intake, indicating rumen degradation of protein (Quigley and Bernard 1992). Because supplementation had no effect on starter intake, no effect was observed for PUN. However, as calves aged and increased starter intake, PUN concentration was also increased, as it occurred with BHBA concentrations (Figure 3). Studies have shown increasing values of PUN in calves receiving diets with different values of crude protein (Khan et al. 2007; Hill et al. 2010); however, the increase of about 1.5% as a result of the supplementation may have been not enough for this effect. As regard to the TSP, there was first a reduction from the second to the fourth week of age, probably as a response of reducing Ig concentrations, but an increase thereafter probably because of increased intake. According to Hill et al. (2010), the main serum constituents used as markers for body mass are creatinine and albumin, while alkaline phosphatase concentration allows the evaluation of bone growth. Albumin concentration is slightly variable as long as the animal is healthy, and no variation was observed according to amino acids supplementation (Table 3). In the present study, alkaline phosphatase

Table 4. Blood metabolites of dairy calves receiving the control or the milk replacer supplemented with lysine and methionine in combination with 0.6% or 1% of Aminogut.

| Treatments | Lys and Met |
|-----------------|-------------|
|                   | Control | Aminogut 0.6% | Aminogut 1% | SEM  | T  | A  | T×A |
| Glucose, mg/dL   | 87.16   | 89.46       | 89.28       | 2.35  | .74 | <.05 | .26 |
| BHBA, mmol/L     | 0.09    | 0.09        | 0.09        | 0.005 | .77 | <.05 | .97 |
| Total protein, g/dL | 5.64   | 5.41        | 5.71        | 0.11  | 18  | <.05 | .58 |
| PUN, mg/dL       | 12.46   | 12.81       | 13.15       | 0.28  | 24  | <.05 | .75 |
| Albumin, g/dL    | 2.58    | 2.58        | 2.58        | 0.05  | 99  | <.05 | .16 |
| Alk. phosphatase, U/L | 127.5  | 122.8       | 133.7       | 9.08  | 69  | <.05 | .49 |

Notes: Control, basal diet without supplementation with amino acids; Lys, Met, Aminogut 0.6%: milk replacer supplemented to meet the requirement of Lysine (17 g/d) and Methionine (5.3 g/d), and addition of 0.6% Aminogut containing 10% Glutamate and 10% Glutamine; Lys, Met, Aminogut 1%, milk replacer supplemented to meet the requirement of Lysine (17 g/d) and Methionine (5.3 g/d), and addition of 1% Aminogut containing 10% Glutamate and 10% Glutamine; SEM, Standard error of the mean; T, treatment effect; A, age effect (week); T×A, interaction Treatment × Age.
increased during the pre-weaning phase, regardless of amino acids supplementation, suggesting bone growth associated with weight gain. Corporal measures corroborate this suggestion since there was an age effect \((P < .05)\) for withers height, heart girth and hip width.

**Conclusion**

Supplementation of milk replacer with lysine and methionine and the association with glutamate and glutamine had no effect on performance, faecal scores or metabolism of calves. The starter intake is the factor that affects performance and metabolism of calves the most and practices that result in its increase should be pursued.

**Disclosure statement**

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