

# Effects of Enteral Fluid Therapy in Continuous Flow Administered by Nasogastric Tube in Buffalo Calves

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**Abstract:** To investigate the employment of enteral fluid therapy in continuous flow administered by a nasogastric tube in buffalo calves; to assess the effects of a hypotonic and an isotonic electrolyte solution on: vital functions, blood count and serum and urinary biochemistry profile of buffalo calves. Seven buffalo calves, clinically healthy, were submitted to two treatments. The solutions were administered in continuous flow through a nasogastric tube at a dose of 15 mL/kg/hr for 12 hours. The serum biochemistry profile showed an increase in chloride concentration, decrease in serum urea and osmolarity. In urine, an increase in sodium and chloride concentrations and a decrease in calcium, creatinine and urea were observed. Enteral fluid therapy in continuous flow proved to be easy to use and effective in maintaining volemia and concentration of electrolytes in buffalo calves.

**Keywords:** Dextrose, maltodextrine, electrolytes, biochemistry, hydration.

## INTRODUCTION

Electrolyte and acid base imbalances are common findings in ruminants' medicine, being associated to a variety of diseases with varying degrees of intensity [1]. The correction of those imbalances is achieved by fluid therapy which, in addition to replenishing fluids and electrolytes, stimulates the return of tissue perfusion and cellular activity that once was decreased [2].

Intravenous and oral administrations are widely used for the replacement of fluid and electrolytes in ruminants. The intravenous route enables a rapid infusion of the replacement volume, being always necessary in cases of severe dehydration and hypovolemic shock [3]. In enteral fluid therapy, although the orogastric probe is highly widespread, the access through the nasogastric route has drawn attention from researchers. This route allows the infusion of large amounts of fluids in a slow and continuous flow, reducing considerably the stress caused by the high number of probe reintroductions that are necessary when the rehydration is performed orogastrically. When using enteral fluid therapy, the

physiological pathway of fluid absorption is maintained. Therefore, the use of sterile solutions is unnecessary, since the mucosa itself serves as a selective barrier [4, 5]. However, the greatest challenge in the employment of this route is to develop a solution that meets the electrolyte demands of dehydrated ruminants. It is known that these solutions should contain sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), chloride ( $\text{Cl}^-$ ), magnesium ( $\text{Mg}^{2+}$ ), calcium ( $\text{Ca}^{2+}$ ) and an energy source, so in cases of anorexia or when the supply of milk must be suspended for calves, the onset of hypoglycemia can be avoided [1].

Many therapeutic protocols established in cattle are applied in the buffalo species without success. This outcome is most likely caused by the lack of knowledge of buffalo's particular physiology when facing a disease process. Currently, experimental studies regarding the use of the nasogastric route in buffaloes cannot be found. Therefore, an essay evaluating this route of fluids administration, the composition of solutions, as well as related risk factors ought to be performed.

Thus, this study aims to investigate the use of enteral fluid therapy (EFT) in continuous flow administered by a nasogastric tube and to assess the effects of a hypotonic and an isotonic electrolyte solution on vital functions, blood count and serum and urinary biochemistry profile of buffalo calves.

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## MATERIAL AND METHODS

### Location and Handling of Animals

The experiment was conducted in the Vivarium Unit of Dairy Bubalino cultura Eva Daher Abufaiad, at the Federal Rural University of Amazonia (BUBali / Ufra). Seven buffalo calves were used (three males and four females), all aged between 45 and 50 days, weighing amidst 40 and 70 kg, clinically healthy, kept in individual, covered stalls, with adequate ventilation, where they received food and water *ad libitum*. Twice a day (early morning and late afternoon) the animals were placed next to their mothers to suckle at will, according to the handling of animals adopted. The experiment was approved by the Ethics Committee for Animal Use in Experiments of the Federal Rural University of Amazonia (Protocol 022/2014).

### Experimental Design

A week before the experiment, all the animals were clinically evaluated (blood count and stool examinations) and then dewormed with sulfate albendazole (Ourofino Animal Health, São Paulo, Brazil). The order of treatment was randomly determined, with a five-day interval between treatments. On the day of the experiment, a halter was placed on animals to help in their contention, in order to avoid too much stress during the probe passage and the collection of biological material. For fluid therapy, a probe of 4.5 mm in diameter and one meter in length was used (PROVAR veterinary products, São Paulo, Brazil), connected to a spiral equipment (Jorgesen Laboratories, Loveland, CO, USA). These were coupled to a 20-liters gallon, positioned one meter above the animals' head, so that the calf could walk and lie down comfortably in the stall. The treatments applied were: HipoMalt - hypotonic electrolyte solution containing 5g of sodium chloride (SulfalQuímicaLtda, Belo Horizonte, MG, Brazil.), 1g of potassium chloride (SulfalQuímicaLtda, Belo Horizonte, MG, Brazil), 2g of sodium acetate (Labsynth, Diadema, SP, Brazil) and 10g of maltodextrin (New Millen Food Products, Cajamar, SP, Brazil) diluted in 1,000 mL of water (Measured osmolarity: 246 mOsm / L); IsoDext - isotonic electrolyte solution (SE) containing 5g of sodium chloride (SulfalQuímicaLtda, Belo Horizonte, MG, Brazil.), 1g of potassium chloride (SulfalQuímicaLtda, Belo Horizonte, MG, Brazil.) 2g of sodium acetate (Labsynth, Diadema, SP, Brazil) and 10g of dextrose (New Millen Food Products, Cajamar, SP, Brazil) diluted in 1,000 mL of water (Measured osmolarity : 293 mOsm/L).

The solutions were administered during a period of 12 hours in continuous flow with a 15 mL/kg/h infusion rate, according to previous studies [3]. During this 12 hour period, the calves remained in fasting with no food or water. The vital parameters measured were heart and respiratory rate, rectal temperature, abdominal circumference and body weight.

Blood samples were obtained by jugular venipuncture, using the vacuum collection system BD Vacutainer (Becton, Dickinson and Company, São Paulo, Brazil), with 25 x 8 mm needles for multiple collection. Blood samples for complete blood count were collected in vials containing K3-EDTA as an anticoagulant and analyzed on an automatic analyzer BC-2800vet (Mindray, Shenzhen, Guangdong, China) and differential count of white blood cells made in blood smears stained with fast Panotic LB (Laborclin, Pinhais, PR, Brazil) in an Olympus CX31 microscope (Olympus, Tokyo, Japan) at 100x magnification.

For biochemical analysis, samples were collected in vials with and without anticoagulant (sodium fluoride), spun in a centrifuge Excelsai 2206 (Fanem, Guarulhos, SP, Brazil), 3000g for 15 minutes, then aliquoted and kept frozen at -70 °C until the time of analysis.

In serum samples, the Na<sup>+</sup> and K<sup>+</sup> analysis were performed by flame photometry in the photometer B462 model (Micronal, São Paulo, SP, Brazil). Through commercial kits, in automatic biochemical analyzer HumaStar 300 (Human, Wiesbaden, Germany), Cl<sup>-</sup> was determined by the colorimetric method of thiocyanate mercury (Life Biotechnology, Belo Horizonte, MG, Brazil); total calcium (tCa) was determined by the colorimetric method of the cresolphthaleincomplexone (InVitroDiagnostics Ltda., Itabira, MG, Brazil); Mg<sup>2+</sup> by the method of xilidila blue (InVitroDiagnostics Ltda., Itabira, MG, Brazil); phosphorus by UV phosphomolybdate (InVitroDiagnostics Ltda., Itabira, MG, Brazil); creatinine by the alkaline picrate colorimetric test (InVitroDiagnostics Ltda., Itabira, MG, Brazil); and serum protein by the Biuret method (InVitro diagnostic Ltda., Itabira, MG, Brazil); glucose and lactate were determined in plasma by means of enzymatic colorimetric tests without deproteinization (InVitroDiagnostics Ltda., Itabira, MG, Brazil) and enzymatic UV using lactate dehydrogenase (Bioclin / Quibasa basic chemistry, Belo Horizonte, MG, Brazil), respectively; and serum urea was determined by enzyme kinetic method of glutamate dehydrogenase (In Vitro Diagnostics Ltda., Itabira, MG, Brazil). Finally,

the serum osmolarity was determined by the freezing point depression method using Model 3320 osmometer (Advanced Instruments, Norwood, MA, USA). All were performed according to the manufacturers' recommendations.

Evaluation of vital signs and blood samples were taken in the following experimental stages: T0h (immediately before hydration); T6h (6 hours of hydration); T12h (12 hours of hydration, end of treatment); T18h (6 hours after the end of hydration) and T24h (12 hours after the end of the hydration).

The urine was collected in properly sanitized and dried plastic containers, picking up all volumes excreted when the animals urinated. The urine samples were frozen at -70 °C for later analysis of Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, tCa, Mg<sup>2+</sup>, glucose, creatinine and urea, through the same analytical procedures and commercial kits used for blood chemistries as the manufacturers' recommendations. After collection, the volume was measured in a beaker, the pH determined by Insight dipstick (Acon Laboratories, San Diego, CA, USA) and urine specific gravity in Master SUR / NM refractometer (Atago CO., Bellevue, WA, USA). For urinary biochemistry, the results were grouped into 6-hour periods: T0: 0 to 6h; T1: 6 to 12h (corresponding to hydration phase); T2: 12 to 18h and T3: 18 to 24h (corresponding to clinical observation phase).

### Statistical Analysis

The data was subjected to normality analysis through the Lilliefors test, and to a homogeneity analysis through the Cochran and Bartlett tests, to

verify ANOVA's assumptions, based on planning the repetition of measures in a certain amount of time, checking the effects of treatment and the time. The contrast was made by the least significant difference on Duncan's tests, when the coefficient of variation was > 15%, or Tukey's test, when the coefficient of variation was ≤ 15%.

When the data did not meet the requirements of ANOVA, a non-parametric analysis was used, and the averages, in time, were compared using Kruskal-Wallis test and the comparison between groups was made using the Wilcoxon test. All analyzes were interpreted considering the significance level of 5% of error probability (P < 0.05). The statistical program SAEG (SAEG-UFV 9.0) was used for data analysis.

### RESULTS

There was no difficulty concerning the passage and setting of the small nasogastric probe in buffalo calves. No significant changes were observed in vital parameters (Table 1). Blood count showed differences (P < 0.05) in the number of red blood cells among the groups in T0h. The HipoMalt treatment significantly promoted an increase in this parameter in T12h when compared to T0h, while the IsoDext treatment produced a difference between T6h and T24h (Table 2). The percentage of eosinophils differed (P < 0.05) between groups in T24h, being the smallest value found on the HipoMalt group (Table 3).

In blood chemistry, shown in Table 4, no significant changes were observed in the concentrations of

**Table 1: Mean Values and Standard Deviation of Physiological Parameters Observed in Buffalo Calves under Two Enteral Fluid Therapy Protocols via Nasogastric Tube in Continuous Flow**

| Parameter   | Treatment | Time evaluation |           |           |           |           |
|-------------|-----------|-----------------|-----------|-----------|-----------|-----------|
|             |           | T0h             | T6h       | T12h      | T18h      | T24h      |
| HR<br>(bpm) | HipoMalt  | 85.7±35.1       | 76.0±30.0 | 74.0±23.1 | 76.0±21.4 | 82.8±30.7 |
|             | IsoDext   | 79.4±23.2       | 85.7±11.0 | 85.1±12.6 | 92.8±8.8  | 87.7±11.9 |
| RR<br>(mpm) | HipoMalt  | 38.9±24.5       | 32.6±27.8 | 30.6±26.4 | 32.3±26.8 | 30.3±23.6 |
|             | IsoDext   | 37.7±33.4       | 26.3±16.9 | 20.6±4.3  | 20.6±5.4  | 25.7±15.6 |
| RT<br>(°C)  | HipoMalt  | 38.7±0.7        | 39.1±0.3  | 38.5±0.3  | 38.4±0.5  | 38.6±0.2  |
|             | IsoDext   | 39.0±0.3        | 39.1±0.3  | 38.7±0.6  | 38.9±0.2  | 38.7±0.3  |
| AC<br>(cm)  | HipoMalt  | 100.3±6.1       | 104.2±4.8 | 106.4±4.9 | 102.8±3.8 | 100.1±3.4 |
|             | IsoDext   | 102.4±4.6       | 105.7±6.8 | 107.3±5.2 | 104.6±5.0 | 100.9±4.0 |
| BW<br>(kg)  | HipoMalt  | 53.1±7.4        | 54.7±7.0  | 59.3±6.3  | 56.5±5.1  | 54.7±6.1  |
|             | IsoDext   | 54.0±8.8        | 56.3±8.5  | 59.5±9.6  | 59.0±9.0  | 55.4±8.3  |

HR = heart rate; RR = respiratory rate; RT = rectal temperature; AC = abdominal circumference; BW = body weight. (P > .05).

**Table 2: Mean Values and Standard Deviation of the Erythrogram of Buffalo Calves under Two Enteral Fluid Therapy Protocols via Nasogastric Tube in Continuous Flow**

| Parameter              | Treatment | Time evaluation         |                         |                        |                        |                        |
|------------------------|-----------|-------------------------|-------------------------|------------------------|------------------------|------------------------|
|                        |           | T0h                     | T6h                     | T12h                   | T18h                   | T24h                   |
| RBC<br>( $10^6/mm^3$ ) | HipoMalt  | 9.3±0.4 <sup>bb</sup>   | 10.3±0.5 <sup>baA</sup> | 10.4±0.7 <sup>aA</sup> | 9.9±0.6 <sup>baA</sup> | 9.5±0.9 <sup>baA</sup> |
|                        | IsoDext   | 10.2±0.6 <sup>abA</sup> | 10.5±0.7 <sup>aA</sup>  | 9.9±0.7 <sup>abA</sup> | 9.7±0.8 <sup>abA</sup> | 9.3±0.5 <sup>baA</sup> |
| HGB<br>(g/dL)          | HipoMalt  | 12.9±1.8                | 14.0±0.8                | 14.1±1.1               | 13.2±1.5               | 12.1±2.8               |
|                        | IsoDext   | 14.1±0.5                | 14.2±1.7                | 13.5±0.8               | 13.2±1.0               | 12.7±1.2               |
| HCT<br>(%)             | HipoMalt  | 44.8±5.0                | 45.9±4.5                | 46.1±3.6               | 44.3±4.8               | 41.4±5.3               |
|                        | IsoDext   | 45.8±6.2                | 47.3±5.6                | 44.9±5.1               | 43.6±4.6               | 42.1±5.4               |
| MCV<br>(fl)            | HipoMalt  | 45.5±6.2                | 46.5±6.5                | 45.4±6.8               | 44.9±7.2               | 45.6±6.5               |
|                        | IsoDext   | 45.0±6.9                | 45.4±6.6                | 45.3±6.5               | 45.2±6.5               | 45.2±6.6               |
| MCHC<br>(g/dL)         | HipoMalt  | 28.8±2.2                | 30.8±3.2                | 30.7±3.2               | 29.9±3.6               | 29.1±5.4               |
|                        | IsoDext   | 31.2±3.9                | 30.3±3.6                | 30.4±3.3               | 30.5±3.7               | 30.4±3.7               |
| MCH<br>(pg)            | HipoMalt  | 13.0±1.5                | 14.2±1.2                | 13.7±1.2               | 13.4±2.0               | 13.2±2.7               |
|                        | IsoDext   | 13.8±0.8                | 13.6±1.0                | 13.6±1.0               | 13.6±0.9               | 13.5±1.0               |
| RDW<br>(%)             | HipoMalt  | 16.9±0.9                | 17.1±0.9                | 16.9±0.6               | 17.0±0.8               | 16.9±0.6               |
|                        | IsoDext   | 17.2±0.8                | 17.3±0.7                | 17.4±0.9               | 17.3±0.8               | 17.3±0.7               |

Means followed by different lowercase letters in the same row and different capital letters in the same column are significantly different ( $p < 0.05$ ).

**Table 3: Mean Values and Standard Deviation of the Leucogram of Buffalo Calves under Two Enteral Fluid Therapy Protocols via Nasogastric Tube in Continuous Flow**

| Parameter              | Treatment | Time evaluation      |                      |                      |                      |                      |
|------------------------|-----------|----------------------|----------------------|----------------------|----------------------|----------------------|
|                        |           | T0h                  | T6h                  | T12h                 | T18h                 | T24h                 |
| WBC<br>( $10^3/mm^3$ ) | HipoMalt  | 17.1±6.0             | 17.7±6.7             | 17.1±5.6             | 18.1±7.0             | 16.7±6.3             |
|                        | IsoDext   | 16.5±5.3             | 17.0±7.0             | 16.5±6.0             | 16.4±6.4             | 16.2±6.3             |
| Neut.<br>(%)           | HipoMalt  | 32.9±7.8             | 32.9±9.0             | 37.0±9.8             | 33.4±6.2             | 38.3±7.6             |
|                        | IsoDext   | 29.4±10.4            | 33.7±6.2             | 31.9±9.8             | 30.7±6.1             | 31.0±7.0             |
| Bas.<br>(%)            | HipoMalt  | 0.9±0.7              | 1.1±0.9              | 0.9±0.7              | 1.0±1.2              | 0.3±0.8              |
|                        | IsoDext   | 1.0±1.2              | 0.9±0.4              | 0.6±0.8              | 1.0±0.8              | 1.0±0.8              |
| Linf.<br>(%)           | HipoMalt  | 63.4±8.7             | 63.6±10.5            | 59.4±10.0            | 64.0±5.0             | 59.7±7.2             |
|                        | IsoDext   | 65.3±9.4             | 63.4±6.8             | 65.4±10.0            | 66.1±6.7             | 64.7±7.1             |
| Mon.<br>(%)            | HipoMalt  | 1.7±1.2              | 2.1±1.5              | 2.6±1.5              | 1.0±1.0              | 1.4±1.0              |
|                        | IsoDext   | 2.6±1.3              | 1.6±1.5              | 2.0±2.1              | 1.9±1.2              | 1.9±1.1              |
| Eos.<br>(%)            | HipoMalt  | 1.1±1.8 <sup>A</sup> | 0.3±0.8 <sup>A</sup> | 0.1±0.4 <sup>A</sup> | 0.6±0.8 <sup>A</sup> | 0.1±0.4 <sup>B</sup> |
|                        | IsoDext   | 1.6±1.6 <sup>A</sup> | 0.4±0.8 <sup>A</sup> | 0.1±0.4 <sup>A</sup> | 0.3±0.5 <sup>A</sup> | 1.3±1.1 <sup>A</sup> |

Neut. = Neutrophils; Bas. = basophils; Linf. = lymphocytes; Mon. = monocytes; Eos. = eosinophils; Means followed by different lowercase letters in the same row and different capital letters in the same column are significantly different ( $p < 0.05$ ).

$Na^+$  and  $K^+$ , however, there was an increase ( $P < 0.05$ ) in  $Cl^-$  concentration at the end of the hydration period (T12h) in animals of the HipoMalt group, which did not change until the end of the experiment. The same electrolyte was different ( $P < 0.05$ ) between the groups throughout the experiment, except for T6h. The

concentration of tCa showed differences ( $P < 0.05$ ) between groups starting in T6h and keeping the difference until the end of the experiment, with the highest values found in the IsoDext group. The  $Mg^{2+}$  values differed significantly between groups throughout the experiment (T0h-T24h). Phosphorus, creatinine,

**Table 4: Means and Standard Deviations of Blood Electrolytes of Buffalo Calves under Two Enteral Fluid Therapy Protocols via Nasogastric Tube in Continuous Flow**

| Parameter        | Treatment | Time evaluation        |                         |                        |                        |                        |
|------------------|-----------|------------------------|-------------------------|------------------------|------------------------|------------------------|
|                  |           | T0h                    | T6h                     | T12h                   | T18h                   | T24h                   |
| Sodium (mmol/L)  | HipoMalt  | 139.1±3.8              | 141.6±4.2               | 142.4±3.9              | 142.9±2.8              | 144.9±6.5              |
|                  | IsoDext   | 138.6±9.5              | 144.9±6.5               | 143.0±8.8              | 144.7±7.8              | 145.6±7.2              |
| Potassium mmol/L | HipoMalt  | 5.8±1.1                | 5.5±1.3                 | 5.1±1.0                | 5.0±0.7                | 4.8±0.4                |
|                  | IsoDext   | 6.2±1.5                | 5.9±1.3                 | 5.5±0.8                | 5.2±0.7                | 5.6±1.6                |
| Chloridem Eq/L   | HipoMalt  | 90.6±1.6 <sup>bb</sup> | 92.9±1.6 <sup>abb</sup> | 94.0±1.5 <sup>ab</sup> | 94.7±1.7 <sup>aa</sup> | 94.0±2.6 <sup>ab</sup> |
|                  | IsoDext   | 95.1±1.9 <sup>aa</sup> | 96.0±1.5 <sup>aa</sup>  | 97.6±2.6 <sup>aa</sup> | 96.6±1.6 <sup>aa</sup> | 97.4±2.4 <sup>aa</sup> |
| Calcium mg/dL    | HipoMalt  | 13.4±0.7 <sup>A</sup>  | 13.1±0.6 <sup>B</sup>   | 12.7±0.4 <sup>B</sup>  | 12.7±0.5 <sup>B</sup>  | 13.1±0.5 <sup>B</sup>  |
|                  | IsoDext   | 13.8±0.6 <sup>A</sup>  | 14.2±0.4 <sup>A</sup>   | 13.5±0.5 <sup>A</sup>  | 14.1±0.7 <sup>A</sup>  | 14.1±0.4 <sup>A</sup>  |
| Magnesium mg/dL  | HipoMalt  | 2.7±1.0 <sup>A</sup>   | 2.7±0.1 <sup>A</sup>    | 2.7±0.9 <sup>A</sup>   | 2.8±0.1 <sup>A</sup>   | 2.7±0.4 <sup>A</sup>   |
|                  | IsoDext   | 2.5±0.1 <sup>B</sup>   | 2.6±0.1 <sup>B</sup>    | 2.5±0.1 <sup>B</sup>   | 2.5±0.7 <sup>B</sup>   | 2.5±0.1 <sup>B</sup>   |
| Phosphor mg/dL   | HipoMalt  | 6.7±1.1                | 7.4±2.0                 | 6.0±0.7                | 5.9±1.6                | 6.2±1.5                |
|                  | IsoDext   | 7.0±2.0                | 6.6±0.8                 | 7.0±1.0                | 6.0±0.8                | 6.1±0.6                |

HipoMalt (hypotonic solution) and IsoDext (isotonic solution); Means followed by different lowercase letters in the same row and different capital letters in the same column are significantly different ( $p < .05$ ).

serum proteins, glucose and lactate were not influenced by time or treatments over time. The urea concentrations did not differ between groups, however, there was a decrease ( $P < 0.05$ ) in T12h on HipoMalt group and in T18h on the IsoDext, which remained the same until the end of the experiment. Serum osmolarity in the HipoMalt group was significantly reduced in T18h when compared to T12h.

The urinary  $\text{Na}^+$  concentration increased ( $P < 0.05$ ) in the last six hours of hydration in both groups and

remained high until the end of the experiment (Table 5). Only the urinary concentration of  $\text{K}^+$  from animals on the HipoMalt group changed over time. However, the difference was only noticed ( $P < 0.05$ ) during the observation period (T2-T3). Between groups, the difference ( $P < 0.05$ ) was detected solely at the end of the experiment (T3). The concentration of  $\text{Cl}^-$  in urine increased significantly in both groups at the end of the hydration period (T1), remaining higher throughout the observation phase. The tCa in urine showed a reduction ( $P < 0.05$ ) at the end of treatment (T1),

**Table 5: Means and Standard Deviations of Blood Biochemistry of Buffalo Calves under Two Enteral Fluid Therapy Protocols via Nasogastric Tube in Continuous Flow**

| Parameter         | Treatment | Time evaluation          |                          |                         |                         |                         |
|-------------------|-----------|--------------------------|--------------------------|-------------------------|-------------------------|-------------------------|
|                   |           | T0h                      | T6h                      | T12h                    | T18h                    | T24h                    |
| Creatinine mg/dL  | HipoMalt  | 1.2±0.2                  | 1.2±0.2                  | 1.0±0.2                 | 1.0±0.1                 | 1.0±0.1                 |
|                   | IsoDext   | 1.4±0.2                  | 1.4±0.2                  | 1.2±0.2                 | 1.1±0.1                 | 1.1±0.1                 |
| Protein g/dL      | HipoMalt  | 7.0±0.7                  | 7.0±0.6                  | 7.0±0.5                 | 6.9±0.6                 | 6.7±0.4                 |
|                   | IsoDext   | 6.7±0.5                  | 7.1±0.6                  | 6.6±0.5                 | 6.6±0.5                 | 6.6±0.6                 |
| Glucose mg/dL     | HipoMalt  | 112.2±46.4               | 114.7±15.7               | 124.9±17.2              | 121.1±18.3              | 115.4±21.3              |
|                   | IsoDext   | 111.1±26.2               | 108.4±10.8               | 121.9±18.0              | 134.7±23.8              | 117.4±20.8              |
| Lactate mg/dL     | HipoMalt  | 20.6±12.6                | 18.0±8.4                 | 19.0±5.9                | 21.0±9.9                | 16.8±10.2               |
|                   | IsoDext   | 18.5±8.9                 | 21.1±7.0                 | 17.4±6.7                | 17.6±6.0                | 15.6±5.2                |
| Urea mg/dL        | HipoMalt  | 20.3±6.6 <sup>aA</sup>   | 15.9±3.9 <sup>abA</sup>  | 13.9±4.9 <sup>ba</sup>  | 9.9±3.2 <sup>ba</sup>   | 13.1±7.8 <sup>ba</sup>  |
|                   | IsoDext   | 18.6±3.7 <sup>aA</sup>   | 15.4±4.4 <sup>acA</sup>  | 14.1±5.2 <sup>acA</sup> | 9.9±4.3 <sup>bcA</sup>  | 9.4±2.0 <sup>bcA</sup>  |
| Osmolarity mOsm/L | HipoMalt  | 284.7±2.2 <sup>abA</sup> | 287.3±4.7 <sup>abA</sup> | 291.4±7.3 <sup>aA</sup> | 283.3±3.4 <sup>ba</sup> | 282.4±3.3 <sup>ba</sup> |
|                   | IsoDext   | 284.9±5.8 <sup>aA</sup>  | 288.0±6.8 <sup>aA</sup>  | 289±4.45 <sup>aA</sup>  | 285.0±5.2 <sup>aA</sup> | 285.6±6.7 <sup>aA</sup> |

HipoMalt (hypotonic solution) and IsoDext (isotonic solution); Means followed by different lowercase letters in the same row and different capital letters in the same column are significantly different ( $p < .05$ ).

**Table 6: Mean Values and Standard Deviations of Urinary Electrolytes of Buffalo Calves under Two Enteral Fluid Therapy Protocols via Nasogastric Tube**

| Parameter           | Treatment | Time evaluation          |                          |                          |                           |
|---------------------|-----------|--------------------------|--------------------------|--------------------------|---------------------------|
|                     |           | T0 (0-6h)                | T1 (6-12h)               | T2 (12-18h)              | T3 (18-24h)               |
| Sodium<br>mmol/L    | HipoMalt  | 15.2±11.5 <sup>aA</sup>  | 46.6±34.0 <sup>bcA</sup> | 87.7±41.3 <sup>ba</sup>  | 107.1±35.9 <sup>bdA</sup> |
|                     | IsoDext   | 10.5±8.0 <sup>aA</sup>   | 34.4±22.5 <sup>bcA</sup> | 91.7±23.3 <sup>bdA</sup> | 67.7±27.2 <sup>dB</sup>   |
| Potassium<br>mmol/L | HipoMalt  | 23.6±15.9 <sup>abA</sup> | 22.2±16.0 <sup>abA</sup> | 15.4±7.1 <sup>aA</sup>   | 31.9±13.6 <sup>ba</sup>   |
|                     | IsoDext   | 23.9±15.6 <sup>aA</sup>  | 15.6±9.1 <sup>aA</sup>   | 19.3±8.7 <sup>aA</sup>   | 17.6±14.8 <sup>dB</sup>   |
| Chloride<br>mEq/L   | HipoMalt  | 26.4±19.0 <sup>aA</sup>  | 51.0±34.5 <sup>bcA</sup> | 59.9±25.6 <sup>ba</sup>  | 133.5±85.4 <sup>bdA</sup> |
|                     | IsoDext   | 26.0±16.0 <sup>aA</sup>  | 48.2±32.5 <sup>bcA</sup> | 77.6±29.8 <sup>bdA</sup> | 84.1±47.6 <sup>ba</sup>   |
| Calcium<br>mg/dL    | HipoMalt  | 0.9±1.1 <sup>dB</sup>    | 0.2±0.2 <sup>dB</sup>    | 1.8±3.6 <sup>aA</sup>    | 1.1±2.3 <sup>aA</sup>     |
|                     | IsoDext   | 2.7±3.6 <sup>aA</sup>    | 0.7±1.5 <sup>bcA</sup>   | 1.5±1.5 <sup>aA</sup>    | 0.8±1.0 <sup>acA</sup>    |
| Magnesium<br>mg/dL  | HipoMalt  | 0.7±1.0                  | 0.2±0.2                  | 0.5±1.0                  | 0.5±0.8                   |
|                     | IsoDext   | 1.3±1.6                  | 0.3±0.7                  | 0.5±0.5                  | 0.3±0.4                   |

HipoMalt (hypotonic solution) and IsoDext (isotonic solution). Means followed by different lowercase letters in the same row and different capital letters in the same column are significantly different ( $p < .05$ ).

**Table 7: Mean Values and Standard Deviations of Urinary Biochemistry of Buffalo Calves under Two Enteral Fluid Therapy Protocols via Nasogastric Tube**

| Parameter            | Treatment | Time evaluation           |                           |                           |                          |
|----------------------|-----------|---------------------------|---------------------------|---------------------------|--------------------------|
|                      |           | T0 (0-6h)                 | T1 (6-12h)                | T2 (12-18h)               | T3 (18-24h)              |
| Glucose<br>mg/dL     | HipoMalt  | 2.0±1.9                   | 1.0±1.0                   | 0.5±0.75                  | 1.3±0.8                  |
|                      | IsoDext   | 1.9±1.3                   | 1.0±0.8                   | 1.9±1.4                   | 1.4±0.7                  |
| Creatinine<br>mg/dL  | HipoMalt  | 36.5±39.2                 | 16.7±12.3                 | 20.5±0.7                  | 19.6±9.5                 |
|                      | IsoDext   | 36.9±25.8                 | 16.7±10.7                 | 16.9±10.3                 | 18.4±10.2                |
| Urea<br>mg/dL        | HipoMalt  | 125.6±65.0 <sup>dB</sup>  | 101.9±53.0 <sup>abA</sup> | 64.9±41.5 <sup>ba</sup>   | 90.9±38.4 <sup>abA</sup> |
|                      | IsoDext   | 172.5±47.4 <sup>aA</sup>  | 96.1±52.2 <sup>ba</sup>   | 82.1±59.8 <sup>ba</sup>   | 75.0±40.8 <sup>ba</sup>  |
| Urinary Volume<br>mL | HipoMalt  | 251.1±151.7               | 269.9±130.2               | 279.3±212.3               | 392.1±292.1              |
|                      | IsoDext   | 314.0±180.5               | 297.9±156.8               | 267.8±135.1               | 325.3±148.4              |
| pH                   | HipoMalt  | 7.0±0.6                   | 7.1±0.4                   | 7.1±0.2                   | 7.1±0.6                  |
|                      | IsoDext   | 6.8±0.4                   | 7.0±0.4                   | 7.1±0.4                   | 7.0±0.4                  |
| Specificgravity      | HipoMalt  | 1004.6±3.4 <sup>abA</sup> | 1002.9±2.2 <sup>ba</sup>  | 1004.7±1.9 <sup>abA</sup> | 1005.8±2.0 <sup>aA</sup> |
|                      | IsoDext   | 1004.8±4.3 <sup>aA</sup>  | 1002.7±1.1 <sup>aA</sup>  | 1003.7±1.3 <sup>aA</sup>  | 1004.5±2.1 <sup>aA</sup> |

HipoMalt (hypotonic solution) and IsoDext (isotonic solution). Means followed by different lowercase letters in the same row and different capital letters in the same column are significantly different ( $p < .05$ ).

returning to baseline values (T0) in the first hours after the provision of SE. Urinary magnesium concentrations, glucose and creatinine did not change significantly ( $P > 0.05$ ). In the HipoMalt group a decrease ( $P < 0.05$ ) was observed in the concentration of urea in T2 when compared to T0. In the IsoDext group a reduction in the concentration of the same compound was found in T1. Both cases presented values that were below baseline until the end of the experiment. The urine volume and pH were not

influenced by the treatments over time. The urinary density was not influenced by the treatments, an increase ( $P < 0.05$ ) was only detected between T1 and T3 on the HipoMalt group.

## DISCUSSION

During the fluid therapy stage, the animals walked and laid down inside the stall without presenting any behavior that indicated discomfort to the administered

volume (15 ml/kg/h) or even rejection of the probe, confirming results of vital functions, which were similar to those obtained in cattle [3, 6], goats [7] and horses [8]. It is noteworthy that there are no reports in previous researches of the use of this technique in the buffalo species.

The difference in erythrocytes number observed between HipoMalt and IsoDext treatment, although significant, is devoid of clinical significance, since the values of that variable in both groups were within the hematological reference values for the species studied [9]. Although a decrease in the number of red blood cells and packed cell volume was expected during the hydration period (T6h and T12h), this has not occurred, signaling that, possibly, the volume of electrolyte solutions and the infusion time was not sufficient to cause significant expansion of plasma volume detected by these three variables. Similar results in cattle and calves [6] were obtained with the same volume and duration of electrolytic solutions infusion used in this study.

The percentage behavior of eosinophils observed in T24h is devoid of clinical relevance because, even in the IsoDext group, where the values were higher, they were still in accordance to those reported in scientific researches [10] for healthy animals of same species and age.

The stability in serum concentrations of  $\text{Na}^+$  and  $\text{K}^+$  shows that the compositions of enteral electrolyte solutions were balanced in relation to the concentrations of these two electrolytes. The maintenance of this balance is provided by the element quantity in the electrolyte solution and its retention or excretion through kidneys. When a rehydration electrolyte solution is administrated, it is expected that, in addition to correcting the existing imbalances, it does not produce undesirable changes in blood concentration of electrolytes in the patient [11].

The increase in the concentration of  $\text{Cl}^-$  was possibly due to its presence in the SE composition associated to the osmolarity difference between them. Solutions with lower osmolarity (HipoMalt) are absorbed in larger quantities in the intestine [12]. It should be emphasized that despite the increase in chloride values in all the treatments, they remained in the reference range for buffaloes [13], demonstrating that none of the tested electrolytic solutions caused an imbalance in the concentrations of plasmatic chloride, despite the significant difference observed in animals on HipoMalt treatment.

The maintenance of serum concentrations of sodium and chloride in the normal rate in animals subjected to hydration is important so that there is no appearance of electrolyte and acid base imbalances, or a change in the serum osmolarity, which could lead to modifications in the absorption rate of electrolytic solution.

The decrease in serum tCa concentration may be related to hemodilution [4], in this case more intensely promoted by HipoMalt solution. However, we emphasize that all the values of this study are higher than those reported for bubaline calves [14].

The  $\text{Mg}^{2+}$  serum values are due to individual variations, since even before the SE administration a difference between groups was already observed. These data provide another important finding: the lack of hypomagnesaemia, which can occur when the patient is submitted to enteral hydration for a long time, reinforcing the security of therapy presented here [12, 15].

Much of the phosphorus filtered by the glomerulus is reabsorbed in the renal tubules, in a co-transport system with sodium. This absorption rate can vary with the ingestion of this element in a way that poor diets or those that does not contain iron can induce 100% of tubular reabsorption of such compound. The results of the present study demonstrate that the treatments, even lacking this element in the SE composition, did not induce disorders in this electrolyte, in contrast with studies that reported a significant reduction of the serum phosphorus in horses which were under enteral hydration [16, 17].

Creatinine is notorious as a renal function marker and its debugging can assist in determining the glomerular filtration rate [18]. Equines submitted to intravenous hydration with a solution containing equine albumin showed no changes in serum creatinine, possibly due to the maintenance of glomerular function, similar to the present study [19].

The total serum protein can be used as a parameter for assessing volemia since its value tends to increase in cases of dehydration and to decrease with volemia [20]. The results of this study demonstrate that the enteral hydration in continuous flow did not cause the onset of hypoproteinemia, as can be observed in cases of hyperhydration [18]. In a study testing the enteral hydration in euvoletic adult cattle, no statistical difference for this variable was also observed [6].

The stability of glucose and lactate values shows that the carbohydrate employed in the SE (10 g/L) were sufficient to maintain the energetic balance in calves. In sick calves, when the supply of milk should be reduced or even suspended, it is common the onset of hypoglycemia [21], which was not observed during the 12 hours of hydration. In a study comparing SE with the same energy sources in horses, there was no difference in glycemic rate as well [2]. This demonstrates that the degradation of energy sources through the normal flora of the gastrointestinal tract of calves was minimal, allowing a better use of sugars by animals.

The behavior observed in serum urea concentration suggests that there was a slight expansion of plasma volume [3]. However, this event occurred later in IsoDext animals, due to the increased osmolarity of the solution, since hypotonic solutions seem to be quickly absorbed in the gastrointestinal tract [12]. In a study with humans and rats, hypotonic SE were absorbed in larger quantities in the intestine [22], justifying the findings of the present study.

Despite the behavior of serum osmolarity observed in HipoMalt, the values did not differ from baseline, reducing the clinical significance of the finding. However, this stability indicates that the expanding effects expected did not occur in sufficient strength to change this parameter. The tonicity of body fluids is closely correlated with the sodium content of the body [23], so that the maintenance of sodium concentration was a decisive condition to the results of osmolarity in this research. Similar results are reported in other scientific works [11, 17].

The increase in urinary sodium concentration after six hours of hydration comes from the regulatory activation mechanisms of these electrolytes in the body, such as the release of atrial and brain natriuretic peptides. Minimal changes in plasma volume and/or sodium concentration can activate this system, which decreases the tubular reabsorption of  $\text{Na}^+$ , increasing its excretion [24], justifying the results of this study.

The variations detected in the urinary concentration of  $\text{K}^+$  are devoid of any clinical significance, since there was no difference from the baseline. However, this finding reinforces the assertion that the SE contained satisfactory amounts of this element, since the excretion of  $\text{K}^+$  is proportional to its ingestion [25].

It is known that a considerable part of  $\text{Cl}^-$  filtered in the kidneys is reabsorbed either actively or passively

along with sodium in the renal tubules [26]. In a study that evaluated the fractional excretion of some electrolytes, a strong positive correlation between urinary concentrations of sodium and potassium was found [27], corroborating the data of this study. Thus, we can affirm that the increase in urinary concentration of  $\text{Cl}^-$  is due to the increase of  $\text{Na}^+$  in urine.

The behavior of tCa concentration in urine is due to the stimulation hydration performs on renal perfusion, leading to less concentrated urine formation, which resulted in the dilution of this electrolyte. The intestine is the main organ that controls the input and output of  $\text{Mg}^{2+}$  from the body. However, the kidneys play an important and vital role in this electrolyte equilibrium [28]. In a study with humans, the urinary concentration of  $\text{Mg}^{2+}$  proved to be an early indicator of possible changes of  $\text{Mg}^{2+}$  in the body [29], reaffirming that the treatments used in this study, even not containing  $\text{Mg}^{2+}$  in their formulations and being administered for 12 hours, did not cause imbalance in this electrolyte.

The results of urine glucose showed that there was no glycosuria, attesting animals' healthy renal function, reinforcing the affirmation that the SE's carbohydrates were well used by the animals' bodies in order to prevent the onset of hypoglycemia. The urinary creatinine concentration, confirms that there was a stimulus in renal perfusion induced by enteral fluid therapy, which led to the dilution of this compound in urine. Complementing this finding, urinary levels of urea suggest an expansion of plasma volume, since lower concentrations in urine were observed in the same periods in which the plasma concentration was lower, in other words, the reduction in uremia was not due to the increase of excretion but by an increase in volemia.

An increase in urinary volume during the treatment period was expected, but was not observed. This behavior can be attributed to two situations, the first one is the collection method. Since it was spontaneous urination, the collected volumes were quite disparate, increasing the confidence interval of the averages, reducing the power of the statistical test [30]. The second one suggests that the dose used (15 ml/kg/h) was able to maintain animals' volemia and renal activity under physiological conditions, even when they were subjected to the food and water fasting for 12 hours. Solely the composition of urine was altered during the period of the treatment, the volume did not present any changes.

The urine pH reflects, in many cases, the acid-base status of the body. However, because of compensatory blood mechanisms, this parameter must be evaluated with caution [18] research with horses, the SE containing maltodextrin and dextrose, were able to induce acidúria [31, 32]. The urine pH of ruminants, under physiological conditions, should vary between 5.5 and 8.0 [33], showing that the results of this study are in agreement with the limits of reference. Thereby, we can infer that the treatments (HipoMalt and IsoDext) did not induce the appearance of acid base imbalance. However, the ideal for a more accurate assessment of the influence of the treatments on the acid base balance would be the blood gas monitoring.

A significant decrease in urine specific gravity at the end of the treatment period was also expected, which did not occur. However, we emphasize that the values found in this study are below the values mentioned in the literature for healthy animals of the same species and age [34]. Therefore, possibly because of this, there were no statistical significance in the results.

The enteral fluid therapy through a nasogastric probe in continuous flow is safe and effective in maintaining fluid and electrolyte balance in buffalo calves. The electrolyte solutions containing maltodextrin and dextrose are able to maintain volemia, stimulate the increase of renal perfusion, causing minimal variations in blood count, vital functions and concentration of electrolytes. Further studies in dehydrated animals are needed to better assess the capacity of this technique in the expansion of volemia and in the replacement of electrolytes.

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