Colegio de Postgraduados

Effect of electrolyzed water supplementation in the liquid diet on the growth performance of Holstein calves

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ABSTRACT
Objective: To evaluate the effect of electrolyzed water (EW) supplementation in the liquid diet on energy intake, growth performance, and health of lactating Holstein calves.
Design/Methodology/Approach: Forty calves were randomly assigned to two treatments: T1=control and T2=5 mL of EW (Supra®) per liter of milk. Calves received colostrum after birth and were individually housed in disinfected metal hutches. Liquid feeding lasted for 60 days, and growth was monitored until 100 days of age. Body weight was recorded every 10 days. Milk and starter intake were measured to estimate metabolizable energy intake. Health was evaluated by recording the incidence of diarrhea, pneumonia, and mortality. Data were analyzed using Student's t-tests, repeated-measures mixed models, and Pearson's χ^2 tests ($p<0.05$).
Results: Average daily gain, weight gain, feed conversion, and metabolizable energy intake were similar between treatments ($p>0.05$). The incidence of diarrhea and pneumonia did not differ, and no mortality was recorded.
Limitations on study/Implications: The study was conducted under controlled management and hygiene conditions, which may limit the extrapolation of results to herds with higher sanitary challenges.
Findings/Conclusions: Electrolyzed water supplementation in the liquid diet of Holstein calves did not affect growth, feed efficiency, or health.

Keywords: Holstein calves, starter feed, growth performance, health.

INTRODUCTION

Neonatal calves are a fundamental component of dairy production systems and must be reared under conditions that preserve their health and allow them to express their full genetic potential. During the first weeks of life, calves face critical periods in which any health impairment may have long-term consequences on productivity, including

growth and overall efficiency (Cangiano *et al.*, 2020). In this stage, neonatal diarrhea and respiratory diseases are the main causes of morbidity and mortality, often associated with pathogens such as *Escherichia coli*, rotavirus, and *Cryptosporidium parvum*, as well as with deficiencies in management, hygiene, and colostrum supply (Silva *et al.*, 2024).

Different technologies have been developed to reduce the prevalence of neonatal diseases (Sedky *et al.*, 2025). In the pharmaceutical sector, citric acid and its salts are widely used as antioxidants, antimicrobials, and anticoagulants (Ibeagha-Awemu *et al.*, 2025). The use of *Moringa oleifera* for controlling infections caused by microorganisms has also been documented, showing effective antimicrobial activity (Allam *et al.*, 2024). In the case of water, treatment and disinfection are critical for animal rearing. Electrolyzed water (EW), rich in hypochlorous acid, has demonstrated antimicrobial efficacy in sanitizing facilities and equipment, and it is widely applied in countries such as Japan and Italy in the food and dairy industries (Rebezov *et al.*, 2022; Suárez-Zuñiga *et al.*, 2023; Meghwar *et al.*, 2024).

The increasing global demand for dairy products has forced producers to improve the efficiency of production systems (Brkić & Puvača, 2024). Successful calf rearing is essential to ensure productive replacement animals; therefore, achieving healthy development in early life is a priority (Battaglini *et al.*, 2023). Proper nutrition plays a determining role: milk, colostrum, and quality starter feed promote both growth and rumen development. Starter feed fermentation in the rumen produces volatile fatty acids (VFA), particularly butyrate, which stimulate epithelial proliferation and accelerate functional maturity of the rumen (Elizondo-Salazar, 2013; Diao *et al.*, 2019; Palczynski *et al.*, 2020). Inadequate energy intake may delay growth and puberty, and compromise fertility (Wathes *et al.*, 2014).

In this context, it has been hypothesized that EW, in addition to its potential as a disinfectant agent, could contribute to calf performance and health when incorporated into the liquid diet. Therefore, the objective of this study was to evaluate the effect of EW supplementation in the liquid diet on energy intake, growth performance, and health of Holstein calves during the rearing stage.

MATERIALS AND METHODS

Study area

The study was conducted from November 5, 2020, to January 30, 2021, in a dairy farm located in the municipality of Delicias, Chihuahua, Mexico. The region is classified as semi-arid and is located at an altitude of 1170 m above sea level, between parallels 28° 11' N and meridians 105° 28' W (INEGI, 2009).

Colostrum management

Colostrum was collected from primiparous and multiparous Holstein Friesian cows within the first 24 h after calving. Immediately after collection, density was determined using a Brix refractometer (Model-1221, DeltaTrak®). Colostrum with ≥24.5% Brix (corresponding to 80 mg mL^{-1} of Ig) was pooled until reaching 15 L (one batch). The colostrum was pasteurized at 60 °C for 60 min in a commercial pasteurizer (Rudder®).

After pasteurization, it was packed in vacuum-sealed plastic bags (20×30 cm, 2 L each) and stored at −20 °C until feeding.

Treatments and liquid feeding

Forty calves were randomly selected and separated from their dams at birth. Calves were individually housed in metal hutches previously cleaned and disinfected. Two treatments were applied: T1=control and T2=5 mL of EW (Supra®) per liter of milk. Both treatments were provided until 60 days of age. Colostrum feeding was given in two doses: the first within two hours after birth, and the second between four and seven hours later. Each feeding consisted of 2 L. Each calf represented an experimental unit (n=20 per treatment). During the rearing period, 500 L of pasteurized whole milk were supplied per calf, divided into two daily meals (08:00 and 15:00 h). Fresh water was offered *ad libitum* throughout the study.

Starter feed management

Starter feed (Table 2) was offered *ad libitum* from the first day of life until weaning. It was provided every morning, and additional feed was offered in the afternoon if necessary. Intake was calculated daily as the difference between the amount offered and the refusals. Refusals were weighed each morning using a digital electronic scale (L-EQ 5, Torrey®).

Body weight recording

Calf body weight was recorded every 10 days from birth until 100 days of age using a livestock scale (PG-2000, Torrey®).

Health records

Calf health was evaluated from birth until 60 days of age by recording cases of diarrhea, pneumonia, and mortality. Diarrhea was diagnosed based on fecal consistency: calves

Table 1. Volume of milk offered to Holstein calves supplemented with EW (Supra®).

Trial days	1-7	8-15	16-40	41-56	57-60
Control	6	8	10	8	4
Supra®	6	8	10	8	4

Source: own elaboration with results from the RStudio output. Values expressed as liters offered per calf per day.

Table 2. Ingredients of the starter feed used in calf feeding.

Ingredient	Value	%*
Moisture	Max.	13
Crude protein	Min.	21.50
Crude fat	Min.	3.00
Crude fiber	Max.	8.00
Ash	Max.	7.00

Source: own elaboration according to the manufacturer's label.

with normal feces were considered healthy, whereas those with semi-pasty to liquid feces were classified as diarrheic. Respiratory problems were identified in calves showing nasal discharge, tearing, coughing, and body temperature above 39.5 °C; calves without these signs were classified as healthy.

Metabolizable energy calculation

Metabolizable energy (Mcal) intake was estimated considering whole milk and starter feed consumption. For milk, an energy value of 0.69 Mcal/L was used, based on the average total solids analyzed during the study period. For starter feed, an energy value of 2.83 Mcal/kg of dry matter was applied, according to the manufacturer's formulation. Individual energy intake was calculated by multiplying the volume or mass consumed by these factors

Statistical analysis

Productive performance (average daily gain, weight gain, total energy intake, and feed conversion ratio) was analyzed using Student's t-tests for mean comparisons between treatments. The effect of time on body weight was assessed with a repeated-measures mixed model (lme, AR(1) structure, random intercept by calf). The incidence of diarrhea and pneumonia was compared using Pearson's χ^2 test. All analyses were performed at a significance level of $p<0.05$ using R software (v4.4.3).

RESULTS AND DISCUSSION

Body weight

Calf body weight increased progressively from birth to 100 days of age in both treatments (Figure 1). Growth trends were parallel between groups, with no evident differences in the trajectory of the curves. In general, calves reached body weights above 100 kg at the end of the observation period.

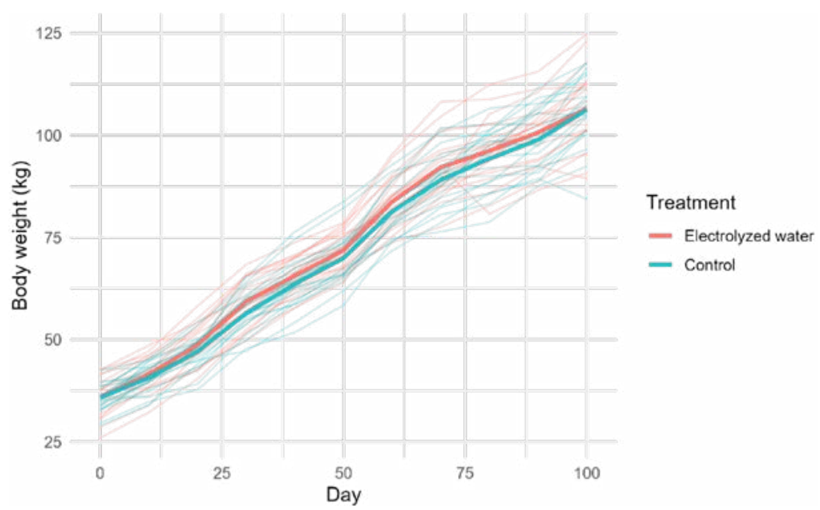

Figure 1. Growth curve of Holstein calves from birth to 100 days of age. Mean values by treatment with standard errors are shown.

At 100 days of age, productive performance was similar between treatments (Table 3). Average daily gain was 0.70±0.10 kg/day, with no statistical difference ($p=0.897$). Total weight gain (~70 kg) and metabolizable energy intake (~71 Mcal) were also statistically equivalent between groups ($p>0.05$). These findings agree with Elizondo-Salazar (2013), who explained that calf energy requirements are directly related to body weight and gain rate, and that adequate intake allows normal growth patterns without compromising health or structural development. Furthermore, early starter intake promotes rumen development and continuous growth. McCurdy *et al.* (2019) demonstrated that consumption of fermentable feed increases volatile fatty acid production, which stimulates rumen epithelial development and facilitates more efficient growth.

Repeated-measures analysis confirmed that body weight increased significantly with time ($p<0.0001$). However, no overall differences were detected between treatments ($p=0.259$), nor a Treatment×Day interaction ($p=0.715$), indicating that growth curves were parallel between groups (Table 4). The lack of interaction suggests that EW supplementation did not alter growth trajectories compared to the control under the feeding conditions of this study. In calves, growth curves during lactation are strongly influenced by milk allowance and the transition to starter feed; higher milk intake often reduces starter consumption before weaning but is compensated after weaning, without affecting overall growth when management is appropriate (Welk *et al.*, 2023). Postnatal rumen development depends on starter fermentation (VFA production and epithelial stimulation), which explains the similar weight curves between groups under equal nutritional supply (Jafari *et al.*, 2020). These results do not support our a priori hypothesis that incorporating EW into milk would enhance growth; with equal milk allowance and starter availability, growth is largely driven by energy intake and rumen development, which were comparable between groups.

Table 3. Productive performance of Holstein calves supplemented with electrolyzed water (EW) or control up to 100 days of age.

Variable	EW	Control	p-value
ADG (kg day^{-1})	0.706±0.110	0.705±0.087	0.961
Weight gain (kg)	70.6±11.02	70.5±8.71	0.961
Total Mcal intake	71.6±7.41	70.9±7.43	0.752

Source: own elaboration with results from RStudio output. Values expressed as mean ± SD. ADG=average daily gain (0-100 days, n=20).

Table 4. Repeated-measures variance analysis for body weight of Holstein calves supplemented with EW or control during 100 days.

Source of variation	Num df	Den df	F
Treatment	1	38	1.315
Day	1	398	10,819.169
Treatment × Day	1	398	0.134

Source: own elaboration with results from RStudio output. Linear mixed model with repeated measures (AR(1) structure, random intercept by calf).

Starter feed intake

During the first 60 days of life, starter feed intake increased progressively in both treatments (Figure 2). The consumption curves were very similar between groups, with no significant differences at any measurement point. At 60 days of age, calves reached average intakes close to 950 g/day.

The absence of differences agrees with reports indicating that, under adequate liquid feeding conditions, starter intake depends more on feed quality and consistency of feeding management than on external factors such as the type of water provided (Gelsinger *et al.*, 2016; Zhang *et al.*, 2019;Nikkhah & Alimirzaei, 2022).

Feed conversion

Feed conversion, expressed as metabolizable energy (Mcal) consumed per kilogram of weight gain, was similar between treatments (Table 5). Calves supplemented with EW showed an FCR of ≈1.02±0.10 Mcal/kg, while the control group reached ≈1.01±0.09 Mcal/kg, with no statistical differences ($p>0.05$).

The efficiency observed indicates that both groups transformed the consumed energy into growth with equivalent yields. These results suggest that EW supplementation did not modify overall energy utilization during rearing. Therefore, this suggests limited room for EW to influence nutrient utilization under these feeding conditions. Similar findings have been reported in studies where liquid diet and starter intake, rather than water source, determine feed efficiency in dairy calves (Wickramasinghe *et al.*, 2019; Jafari *et al.*, 2020).

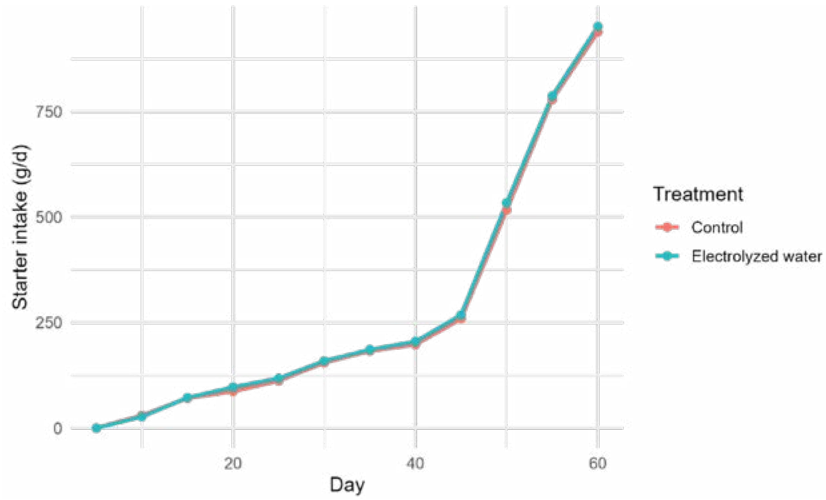

Figure 2. Average starter feed intake (g/day) of Holstein calves supplemented with EW or control during the first 60 days of age.

Table 5. Feed conversion (Mcal kg^{-1} of weight gain) in Holstein calves up to 100 days of age.

Variable	EW	Control	p-value
Feed conversion (Mcal/kg gain)	1.04±0.20	1.04±0.20	1.04±0.20

Source: own elaboration with results from the RStudio output. Values expressed as mean ± SD. FCR=total metabolizable energy intake (Mcal)/weight gain (kg) from 0-100 days.

Likewise, recent studies emphasize that milk feeding consistency and feed quality are the main determinants of FCR, while external factors such as water type show no clear effects on conversion efficiency (Senevirathne *et al.*, 2018; Wickramasinghe *et al.*, 2019).

Health

Health evaluation up to 60 days of age showed a diarrhea incidence of 30.0% in the EW group and 35.0% in the control group, with no statistical differences (p=0.736). Pneumonia occurred in 5.0% of control calves, while no cases were detected in the EW group (p=0.311). No mortality was recorded in either treatment (Table 6).

Table 6. Incidence of health problems in Holstein calves up to 60 days of age.

Variable (%)	EW	Control	p-value
Diarrhea	6 (30.0)	7 (35.0)	0.736
Pneumonia	0 (0.0)	1 (5.0)	0.311
Mortality	0 (0.0)	0 (0.0)	NA

Source: own elaboration with results from RStudio output. Data expressed as frequency (percentage), p-value: Pearson's χ^2 test.

These results indicate that EW supplementation did not significantly reduce the incidence of diarrhea or pneumonia, although a favorable trend was observed in the absence of respiratory cases among treated calves. Neonatal diarrhea remains one of the main health problems in calves, mainly associated with *Escherichia coli*, rotavirus, and *Cryptosporidium parvum*, and with management factors such as hygiene, colostrum quality, and stress rather than the type of water provided (Brunauer *et al.*, 2021). Furthermore, improvements in water microbiological quality can contribute to reducing infection pressure, although effects depend on the initial contamination level and herd management practices (Kamal *et al.*, 2024). The absence of mortality in both groups is consistent with reports from systems where adequate colostrum feeding and hygienic management reduce the impact of enteric and respiratory diseases (Keller *et al.*, 2024).

CONCLUSIONS

Electrolyzed water supplementation in the liquid diet of Holstein calves did not significantly affect growth performance, energy intake, or health indicators during the rearing period. Under adequate management conditions, the use of EW can be implemented without negative effects on calf development; however, it does not provide additional benefits in weight gain or disease reduction during the pre-weaning stage.

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