Effect of the administration of a protected source of calcium gluconate on growth, feed efficiency, nutrient digestibility, and health in beef cattle

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SUMMARY

The study evaluated the effects of a protected source of calcium gluconate on production performance, digestibility, and health in beef cattle. A total of 241 Charolaise bulls were randomly divided into two groups: i) Control (n° 120) basal diets + 10g/head/day of a placebo of wheat bran; ii) Treatment (n° 121) basal diets + 10 g/head/d of a protected source of calcium gluconate. The population of each group was divided into two subgroups: i) "Overall population" (n° 100 Control and n° 101 Treatment) housed in pens of 7/8 animals each; ii) "Beefmonitor population" (n° 20 Control and n° 20 Treatment) housed in two pens (Control and Treatment) equipped with two automatic weighting scales to evaluate the daily trends in production performance. Growth performance, feed intake (FI), feed conversione rate (FCR), were evaluated on both subgroups. Slaughtering performance, apparent total tract digestibility (aTTD) and health were evaluated equally. The average daily gains (ADG) were improved by the Treatment (+40 and +43 g/head/day compared with the Control group, in the "Overall" and "Beefmonitor" populations) (P=0.0084 and <0.0001 respectively). The final weights were also higher (706.82 and 705.83 vs 699.75 and 697.33 kg in the Control group in the "Overall" and "Beefmonitor" populations) (P=0.0084 and 0.037 respectively). The FCR was improved (6.90 and 7.03 vs 7.51 and 7.28 in the Control group in the "Overall" and "Beefmonitor" populations) (P=0.0008 and <0.0001 respectively). The aTTDs of starch (97.05 vs 95.71% in the Control group) (P<0.0001), cellulose (57.91 vs 52.95% in the Control group) (P<0.0001) and NDF (52.02 vs 50.02% in the Control group) (P=0.020) were improved by the Treatment. The incidence of lameness was reduced (0.99 vs 7% in the Control group) (P=0.0282). Including protected sources of calcium gluconate can be functional to improve production efficiency, due to a protective effect on gut health and integrity.

KEY WORDS

Beef cattle; gut health; efficiency.

INTRODUCTION

Currently, the zootechnical sector must move toward a renewed mindset, with environmental sustainability, circular economy, animal welfare and innovation as main focal points, without forgetting the need to feed the growing world population and to sustain the livelihood of farmers and of a multitude of other stakeholders [1,2].

In this light, enhancing production efficiency is becoming thus a crucial key [3,4,5,6]. Higher food production and other environmental and economic benefits can be obtained through an optimized efficiency. Considering pollutants, such as greenhouse gases, emission intensities per unit of final production are reduced because of a higher productivity per animal. Moreover, higher efficiency and productivity rates are often determined by a better digestive function, allowing a more complete conversion of feeds into valuable nutrients, instead of side wastes, such as methane [2,7]. Also, inputs (e.g. feed materials, and others such as energy, gasoline etc.) needed per unit of final product can be reduced if the efficiency of their conversion is maximized, with economic, social, and environmental improvements [8]. Feed costs and yardage costs can be reduced, improving the profit for the farmer [4]. Increasing the production efficiency can also be a step forward in terms of ethical development of the system. To maximize the productivity, it is thus crucial to improve and ameliorate animal well-being, satisfying thus the consumers' demand for better welfare, also tackling the issue of antimicrobial resistance by improving the overall animal health and resilience [8,9].

Thus, the development, implementation, and application of innovative strategies to improve and boost production efficiency is fundamental.

The gastrointestinal tract (GIT) has a pivotal role in determining productivity and efficiency in both monogastric and ruminants [10]. Indeed, besides being involved in the digestion and absorption of nutrients, the GIT is reported to be deeply involved in different processes and functions related to the inflammatory status, the hormone release, the immune and even the nervous systems [10,11]. It also hosts a complex microflora of both benign and commensal microbes, that however can become dangerous in critical and unbalanced situations, leading thus

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to local and metabolic issues and diseases [12,13]. Also, the GIT represents the main interconnection, and even barrier, between the animal, the feeds, the external environment, and the gut microflora. It manages thus their exchanges and acts as a protection against some possible external attack, such as bacteria, viruses, and toxins [14]. A correct GIT health and functionality are needed to safeguard animal productivity and health [10].

In ruminants, a great attention was placed over the years on ruminal functionality and safety, due to its complexity, its complicated microflora, and its centrality in feed digestion. Many scientific studies have been performed on the role of ruminal alteration and unbalances, and their negative effects on animal production and health in both dairy and beef cattle, with a greater attention in the transition phase in dairy cows and on high-starch diets in fattening beef cattle. The negative effects of clinical and subclinical ruminal acidosis on both feed efficiency, productivity and overall animal health are well-known [15,16,17].

Conversely, the role of hindgut, which consists of the cecum, colon, and rectum, in ruminant nutrition has received little scientific attention. However, some recent insights have highlighted a stronger role of it on both animal performance and health, with a specific focus on some systemic issues normally correlated with ruminal acidosis such as lameness and inflammation [18,19,20]. In the lights of those recent findings, it is indeed believed that those issues might be caused more likely by an impaired hindgut function [19]. Indeed, the administration of high-starch diets can lead to a higher flow of it in the hindgut, with negative effects on its environment (e.g. pH reduction), that are likely comparable to those in the rumen, that are caused by an increased carbohydrate fermentation by the large intestine microflora [20]. However, the hindgut is believed to be less resistant to acidotic conditions compared to the rumen, mainly because of differences in the anatomical structure of its epithelium and of lower buffering potential [18,20,21]. Those conditions can cause epithelial damage, reducing thus the integrity of the intestinal barrier and promoting the onset of a proinflammatory status at the local and systemic levels. Indeed, an impaired integrity of the hindgut barrier can cause the transmigration of different problematic compounds, such as antigens, toxins and even bacteria, from the hindgut lumen to the blood stream. This phenomenon is known as "leaky gut" [20]. Those compounds exert negative systemic effects, inducing an inflammatory condition, with also some specific targets, such as limbs and liver, causing liver abscesses and laminitis [22,23]. Thus, the evaluation and potential application of innovative nutritional strategies aimed at protecting the hindgut and maximizing its resilience toward acidotic conditions is crucial in beef and dairy cattle farming.

In this light, the inclusion of protected forms of calcium gluconate, that reach and act at the hindgut level, can be functional. Indeed, gluconic acid is slowly fermented by lactic acid bacteria, such as *Lactobacillus reuteri* and *Lactobacillus mucosae*, into lactate and acetate that, after, are converted to butyrate by acid-utilizing bacteria, such as *Megasphaera elsdenii* and *Mitsuokella multacida* [24,25]. Through this mechanism, protected calcium gluconate induces a higher production of butyrate and decrease lactate at the gut level. It has been demonstrated that butyrate can have multiple beneficial effects on gastrointestinal ecology, morphology, and function, stimulating as an example of the proliferation of epithelial cell and improving thus the gut barrier function, especially in monogastric animals and pre-weaned ruminants, with positive effects also on gastrointestinal health and production performance [24,26,27]. Also in adult ruminants, the role of butyrate on epithelium development is well-recognized at the ruminal level, while the mechanism of actions at the gut level are still to be clarified [28]. However, there is different scientific evidence that underlines some positive roles of the administration of protected forms of gluconate to high-producing dairy cows, on production performance and milk composition, such as fat content. Those results can be mainly ascribed to a change in the volatile fatty acids (VFA) production at the hindgut level, as well as to a protective action against the negative local and systemic side effects of acidosis, that lead to a better health status and to a greater availability of energy for production instead of immunity [28,29,30,31].

Considering beef cattle, a higher VFA concentration in hindgut digesta of growing steers were detected, as well as some differences in the microbial population in the cecum and colon toward more beneficial and efficient colonies in animals that received a protected form of gluconate [32,33]. However, there is still a lack of scientific evidence about the role and effects of the administration of protected sources of gluconate in beef cattle on health, production efficiency and growth performances.

Thus, the aim of the study is to investigate the effect of the administration of protected calcium gluconate on health, growth performance, feed efficiency and apparent total tract digestibility in fattening beef cattle from arrival to slaughter.

MATERIALS AND METHODS

2.1. Animals, groups and animal care

The study was carried out at Meneghini Farm (Via Viola 16, Roverchiaretta, Verona - Italy), that well represents the typical specialized beef cattle farms. A total of 241 Charolaise beef cattle bulls, imported from France, were enrolled in the trial at the arrival, and housed on straw bedding in pens of 7 or 8 heads each.

The animals were divided in two experimental groups: i) Control (n° 120) basal feeding plan + 10g/head/day of a placebo of wheat bran; ii) Treatment (n° 121) basal feeding plan + 10 g/head/d of the protected source of calcium gluconate (Lactibute, Trouw, Località Vignetto, 17 - 37060 Mozzecane (VR), Italia). A group of 201 animals (100 per group), were dedicated to a standard performance trial. This group was thus named "Overall population".

A subset of animals included in the trial was dedicated to a growth trends trial, where two weighting scales (BeefMonitor, Ritichie Agricoltural, Carseview Road, For-far, Scotland, DD8 3BT) were mounted on the drinking troughs to weights the animals every day, multiple time per day. The general aim was to evaluate the growth curves, and, in combination with feed intake data, the overall production efficiency trends. This group of 40 animals (20 animals per group), was thus named "Beefmonitor population". Those animals were ranked and divided into the Control and Treatment groups based on body weight and conformation scores (1: profiles from straight to slightly concave and muscle development between medium and good; 2: overall convex profiles and good to very good muscle development; 3: all convex profiles and excellent muscle development), in order to have a comparable population. Those an-

imals were housed in two pens equipped with the two automatic weight scales mounted on the drinking trough.

2.2. Nutritional management

All the animals were raised under the same feeding plan, reported in Table 1. The nutritional management consisted in three different diets, an arrival diet fed from day 0 to day 19, a fattening diet fed between day 20 to day 99 and a finishing diet fed between day 100 to slaughter at day 186. The feeding plan was developed to satisfy the needs of fattening beef cattle in the different stages of life [34]. All the diets were delivered ad libitum in the form of total mixed ration (TMR).

The two groups differed only for the inclusion of the treatment product (Lactibute-Trouw, Località Vignetto, 17 - 37060 Mozzecane (VR), Italia) and of a placebo constituted of wheat bran, in the mineral and vitamins mixes of the two groups as follow:

1. Treatment: Basal diets supplemented with 10 g/head/d of the protected source of calcium gluconate.
2. Control: Basal diets supplemented with 10 g/head/d of wheat bran as placebo.

Both the treatment and placebo were included in the two mineral and vitamins mix used in the two groups to optimize the distribution in the TMR. Lactibute is a rumen protected blend of hydrogenated refined palm fat (55%), calcium gluconate (40%) and calcium carbonate (5%).

2.3. Experimental parameters

2.3.1. Production performance

All the animals in both the "Overall population" and "Beefmonitor population", were individually weighted on day (d) 3 and 186 after arrival and the average daily gain ($ADG_{3\text{-}186}$) was than calculated.

Furthermore, the animals in the "Beefmonitor population" were weighted automatically multiple times per day by the two automatic weight scales (BeefMonitor, Ritichie Agricoltural, Carseview Road, Forfar, Scotland, DD8 3BT). The daily average weights were then calculated as a daily average of all the measurements, after the elimination of outliers' data, mainly caused by incorrect positioning of animals on the scale. The daily gain was then calculated as a difference between the average weights of two consecutive days.

The feed intake (FI) was monitored on all the animals. In the two "Beefmonitor population" pens, the group FI was monitored weekly, by evaluating the fresh feed administered per pen and the refusal after 24h. Considering the "Overall population" pens, the group FI was monitored once a month by evaluating the fresh feed administered per pen and the refusal after 24h. The feed conversion rate (FCR) was then calculated by comparing the weekly ADG and FI for the "Beefmonitor population" group and the overall ADG and average FI for the "Overall population", between 3 and 183 days after arrival.

At the end of the fattening period (186 days after arrival) all animals were slaughtered. Final live weights (d186), and carcass characteristics, such as carcass weight, slaughtering yield, and carcass conformation and fattening (SEUROP classification) were evaluated individually on each carcass.

2.3.2 Apparent total tract digestibility

Ten pens per group in the "Overall population" group were selected to evaluate the *in vivo* apparent total tract digestibility (aTTD). In those pens, both feed and fecal chemical compositions were evaluated, using a portable NIR system (Polispec, IT Photonics, Italy), along the trial at three different sampling periods (period 1: days 20-21; period 2: days 100-101; period 3: days 180-181), that represents the different nutritional phases, in order to calculate the *in vivo* apparent total tract digestibility (aTTD).

The feed composition was evaluated on each single day in the different sampling periods, as an average of multiple single shot measurements performed along the entire feed bunk of the 10 pens selected in each group. The faecal composition was evaluated daily, in each sampling period, on sample of faeces collected from the same 10 pens per group, on a pool of fresh feces deriving from at least 5 animals per pen.

Diets and faeces samples were analysed for dry matter, crude protein, non-protein nitrogen, lipids, starch, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent (ADL) and ash. Hemicellulose contents were calculated from the difference NDF - ADF. Cellulose contents were calculated from

Table 1 - Diets formulation, as predicted by the rationing software.

	Arrival, d 0-19	Fattening, d20-99	Finishing, d100-185
Corn Silage 350832[1]	8.00	8.00	8.00
Wheat Straw	1.80	1.20	1.20
Corn Meal 72 6[2]	1.50	4.50	6.50
Soybean Meal 44[3]	0.8	1.50	1.50
Minerals and Vitamin mix	0.20	0.2	0.2
As fed, kg	12.30	15.40	17.40
Dry matter, kg	6.61	9.35	11.12
Dry matter, %	53.77	60.70	63.89
UFV, kg d.m.[4]	0.85	1.01	1.05
CP[5], % d.m.	11.33	13.58	12.81
Sugars, % d.m.	5.02	4.59	4.08
Starch, % d.m.	28.67	41.42	46.44
NDF[6], % d.m.	41.95	28.61	25.55
Fats, % d.m.	2.50	2.89	3.04
Ca[7], % d.m.	0.92	0.66	0.56
P[8], % d.m.	0.27	0.32	0.31

[1]Corn silage with 35% dry matter, 8% of crude protein and 32% of starch on dry matter; [2]Corn meal with 72% starch and 6% of crude protein on dry matter; [3]Soybean meal with 44% of crude protein on dry matter; [4]d.m.= dry matter; [5]CP= crude protein; [6]NDF= neutral detergen fiber; [7]Ca=calcium; [8]P= phosphorus

the difference ADF - ADL. Sugars and pectin were calculated from the difference 100 -(ash+lipids+proteins+NDF+starch). The aTTD of ash, fats, sugars, starch and NDF was then calculated for each day in each sampling period. The digestibility of crude protein was not calculated due to the lack of data related to the nitrogen partitioning in urines. The aTTD was obtained from the following equation (1), proposed by [35,36]:

$$aTTD\ \% = \frac{\left(\frac{Xd}{ADLd}\right)-\left(\frac{Xf}{ADLf}\right)}{\left(\frac{Xd}{ADLd}\right)} * 100 \quad (1)$$

Where:
X = each analytical parameter considered (%)
ADL = acid detergent lignin (%)
d = diet
f = faeces.

2.3.3 Health status

All the animals involved in the trial were checked daily by the farm technician and veterinary staff. Any cases of disease, such as digestive disorders, bovine respiratory disease (BRD) and locomotory issues, were recorded, as well as the mortality rate and the number of animals that needed to be moved to the infirmary pen.

2.4. Statistical analysis

Data analysis was conducted using SAS statistical software (SAS 9.4, SAS, Cary, NC, USA).

Data related to production performance were analysed using two different approaches when considering the "Overall population" and the "Beefmonitor population" groups.

Considering the "Overall population" group, live weight, $ADG_{3\text{-}186}$, carcass weights, and slaughtering yields were analysed using the mixed procedure of SAS, using the single animal as experimental unit, and including the fixed effect of the group. Since the starting weights were significantly different, they were used as a covariate. The same procedure was used for the initial and final weights in the "Beefmonitor population". Feed intake and feed conversion rate were calculated using the same procedure but using the pen as statistical unit.

Considering the "Beefmonitor population" subgroup, all the daily weigh measurements were checked for outliers and then averaged to obtain the average daily weights for each animal. The weekly averages were then calculated for each animal. Then, the weekly averages were analysed using a mixed model for repeated measures, that account for the effects of the treatment, time, and their interaction, using the single subject as statistical unit. Similarly, the weekly FI and FCR were analysed using a mixed model for repeated measures, that account for the effects of the treatment, time, and their interaction.

The aTTD was monitored for each single day included in the three different sampling periods (day 21, 21, 100, 101, 180, 181) and then the average of each period was calculated (phase 1: days 20-21; phase 2: days 100-101; phase 3: days 180-181). Those data were analysed using a mixed model for repeated measures that accounted for the effect of treatment, time, and their interactions.

For non-continuous variables such as SEUROP classification, fattening score, and health status, the difference in frequency distribution within classes was assessed by applying a chi-squared test.

A difference was considered significant for $p \leq 0.05$, while a tendency toward significance was set at $p<0.1$.

RESULTS

3.1. Production performance

Data related to the production performance of the "Overall population" are reported in Table 2. Since the initial weights were significantly different (411.67 kg in the Treatment group vs 422.04 kg in the Control group) (P=0.0005), they were used as covariate.

Table 2 - Growth performance registered in the "Overall population" group.

	Control	Treatment	P Value
Weight, kg			
d3	416.83	416.83	-
d186	699.75	706.82	0.0084
$ADG_{3\text{-}186}$ [1], kg/head/d 0-186	1.52	1.56	0.0084
FI[2], kg d.m.[3]	11.70	11.60	0.4819
FCR[4]	7.51	6.90	0.0008

[1]ADG= average daily gain, kg/head/d; [2]FI= feed intake, kg; [3]d..m. = dry matter; [4]FCR= feed conversion rate

Table 3 - Slauguthering performance in the "Overall population" group.

	Control	Treatment	P Value
Carcass hot weight, kg	418.38	422.31	0.0594
Dressing percentage, %	59.78	59.75	0.765
SEUROP			
Cat. E, % (n)	79.59 (78)	81.00 (81)	0.879
Cat. U, % (n)	20.41 (20)	19.00 (19)	0.879
Fatness			
Cat. 2, % (n)	51.02 (50)	53.00 (53)	0.887
Cat. 3, % (n)	48.98 (48)	47.00 (47)	0.887

Table 4 - Individual starting and final weights and slaughtering performance in the "Beefmonitor population".

	Control	Treatment	P Value
Initial weight d3, kg	417.35	417.50	0.969
Final live weights d186, kg	697.33	705.83	0.037
ADG_{3-186}, kg/head/d[1]	1.507	1.550	<0.0001
FI[2], kg d.m.[3]	10.52	10.48	0.9994
FCR[4]	7.28	7.03	<0.0001
Carcass hot weight, kg	417.15	421.66	0.1850
Dressing percentage, %	59.81	59.73	0.6060
SEUROP			
Cat. E, % (n)	75.00 (15)	80.00 (16)	0.2747
Cat. U, % (n)	25.00 (5)	20.00 (4)	0.2747
Fatness			
Cat. 2, % (n)	60.00 (12)	55.00 (11)	0.2384
Cat. 3, % (n)	40.00 (8)	45.00 (9)	0.2384

[1]ADG= average daily gain, kg/head/d; [2]FI= feed intake, kg; [3]d..m. = dry matter; [4]FCR= feed conversion rate.

The inclusion of the tested protected form of calcium gluconate in the Treatment group has led to significant improvements in the growth performance, as highlighted by the better average daily gain (ADG_{3-186})(1.56 *vs* 1.52 kg/head/d in the Control group) (P=0.0084), resulting, on average, in an increase of about +40g per head per day. Those improvements have led to a significantly higher average final weight at d186 in the Treatment group (706.82 *vs* 699.75 kg in the Control group) (P=0.0084). Since the feed intake (FI) was unaffected, the feed conversion rate (FCR) was significantly improved by the Treatment (6.90 *vs* 7.51 in the Control group) (0.0008).

Data related to slaughtering performance of the "Overall population" are reported in Table 3. The better growth performance highlighted in the Treatment group have led to a tendency toward significantly higher carcass weights (422.31 *vs* 418.38 in the Control group) (P=0.059).

No significant differences were found in terms of SEUROP and fattening scores between the two groups.

Data related to the average growth and production performance of the "Beefmonitor population" are reported in Figure 1. The initial weights were comparable in the two groups (417.35 vs 417.50 kg of live weight in the Control and Treatment group respectively) (P=0.969).

As visible in Figure 1A, the weekly ADGs were similar till the third week. Starting from the fourth week, the ADGs started to be significantly higher in the Treatment group (1.605 vs 1.564 kg/head/day in the Control group) (P=0.0463). From that point, the ADGs were continuously significantly higher, besides week 11, in the Treatment group till the end of the trial (1.137 vs 1.084 kg/head/day in the Control group in week 27) (P=0.0186). On average, considering the entire trial period, the ADG was significantly higher in the Treatment group compared to the Control, leading to about +43g/head per day (1.550 vs 1.507 kg/head/day in the Control group) (P<0.0001).

However, no significant differences (Fig.1B) were found in terms of weekly individual weights. However, when the final weights at slaughter were analysed separately at d186, the Treatment group showed a significantly higher final live weight (705.83 vs 697.33 kg in the Control group) (P=0.037) (Table 3). The growth trends, as visible in Figure 1A-B, are comparable in both groups, highlighting, starting from week 15, a continuous decline in the growth.

In terms of FI, reported in Figure 1C, no significant differences were recorded both considering the average values (10.48 vs 10.52 kg/head/day in the Treatment and Control group respectively) and the weekly ones. As visible in Figure 1C, the trends in FI were comparable in the two groups, with a significant increase overtime (P<0.0001).

The average FCR was significantly improved by the treatment (7.03 vs 7.28 in the Control group) (P<0.0001), due to the higher ADG coupled with the unaffected FI. The trends in feed conversion efficiency are reported in Figure 1D. The feed conversion efficiency was similar in the two groups till the third week. Starting from week four (6.25 vs 6.52 in the Control group in week 4) (P=0.0041), the Treatment group showed continuatively a better feed conversion rate till the end of the trial (10.25 vs 10.80 in the Control group in week 27) (P<0.0001).

On average, there was a 3.55% improvement in terms of feed conversion efficiency.

The FCR started to worsen similarly in both groups from week 16, as result of the combined increase in feed intake and reduction in the growth performance, as highlighted in Supplementary Figures 1 and 2.

No significant differences were recorded in terms of carcass weights and SEUROP and fattening scores in the Beefmonitor population (Table 4).

3.2. Apparent total tract digestibility (aTTD)

Data related to the chemical composition of the analysed feed and faeces are reported in Supplementary Table 1 and 2. The analyses of the TMRs showed a good similarity with the theoretical nutritional values, given by the rationing software, of the diets of the different phases, as well as a good comparability between the two experimental groups. Considering the faeces, the starch content was lower (on average 6.38 vs 9.01% on dry matter basis in the Control group) in the Treatment group because of the better digestibility highlighted in Table 5.

As visible, no significant differences were recorded in the aTTD of sugars and lipids in all the sampling periods. Conversely, the digestibility of starch was always significantly improved by the Treatment. On average, the digestibility of starch was 97.05% compared to the 95.71% of the Control group (P<0.0001) leading to an average increase of about 1.34 points of aTTD, that is equal to an improvement of about 1.4%.

Also, the digestibility of cellulose was significantly higher in the

Treatment group in the fattening phase (sampling period 2) (55.97 vs 51.58% in the Control group) (P= 0.0054) and during the finishing stage (sampling period 3) (59.56 vs 54.01% in the Control group) (0.0004). On average, the digestibility of cellulose was 57.19% compared to the 52.95% of the Control group (P<0.0001), leading in the end to an average increase of about 4.24 points of aTTD, that are equal to an improvement of about 8%.
Consequently, the total NDF digestibility was also increased in the fattening phase (sampling period 2) (47.38 vs 45.68% in the Control group) (P= 0.057) and during the finishing stage (sampling period 3) (53.84 vs 52.00% in the Control group) (0.0409). On average, the digestibility of NDF was 52.02% compared to the 50.02% of the Control group (P=0.020), leading to an improvement of about 3.98%.
The ash digestibility was significantly lower in the Treatment group in the fattening phase (sampling period 2) (61.93 vs 64.27% in the Control group) (P= 0.01) and during the finishing stage (sampling period 3) (64.96 vs 68.31% in the Control group) (0.0004).

3.3. Health status

Data related to the health issues recorded during the trial are reported in Table 6. No issues were indeed recorded in the animals selected for the "Beefmonitor population" trial. The treatment didn't affect the mortality rate even if two deaths were registered in the Control group due to enterotoxaemia. Also, the number of severe health issues that required the animals to be moved to the infirmary pen, and the incidence of BRD weren't affected by the Treatment.
Conversely, there was significant reduction in the incidence of lameness ascribable to nutritional causes in Treatment group compared to control one (0.99 *vs* 7.00% in the Control group) (P=0.0282).

DISCUSSION

The health and functionality of the overall GIT in livestock have a central role in maximizing production efficiency, as well as of animal wellbeing, resilience, and the overall sustainability of the production system.
In ruminants, a greater attention was placed over the years on ruminal health and balance. However, it is now recognized that the hindgut plays a certain role in both digestion efficiency and overall animal welfare and health, since it is deeply interconnected with the immune functionality, the hormonal release, and the nervous system. Also, it regulates the exchanges and relationship between the animal, the intestinal microflora, and the external environment. Damages and unbalances at this lev-

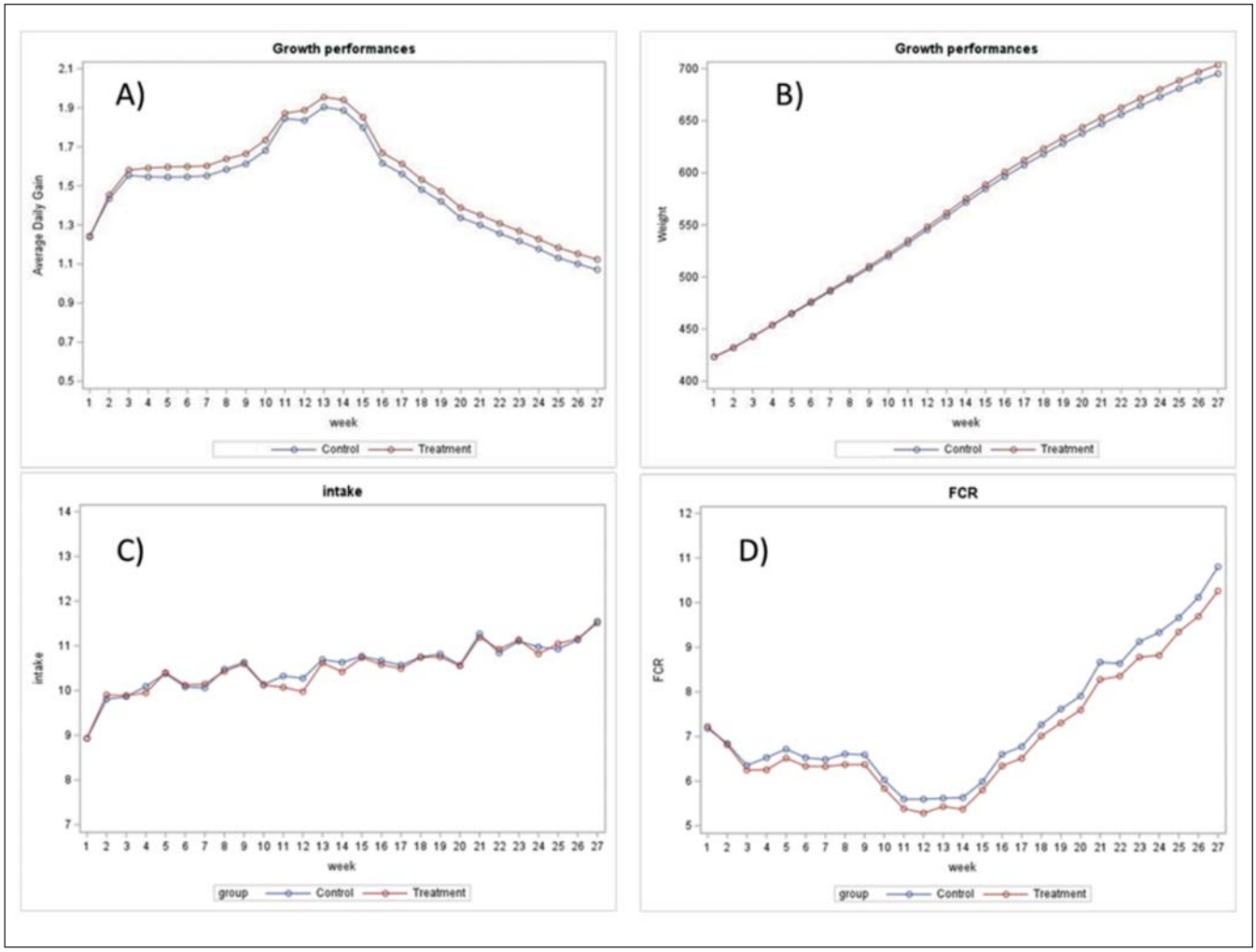

Figure 1 - Trends and differences in the growth and production performance measured in the "Beefmonitor population". Picture A) shows the weekly trends in daily average daily gain (ADG; kg/head/d). Picture B) shows the weekly trends in live weights (kg). Picture C) shows the weekly trends in feed intake (FI, kg/head/day). Picture D) shows the weekly trends in feed conversion rate (FCR).

Table 5 - The apparent total tract digestibility aTTD detected in the different timepoints.

Sampling period	Group		P value
	Control	Treatment	
Sugars digestibility			
1[1]	98.54	98.52	0.8888
2[2]	98.24	98.21	0.8879
3[3]	98.56	9837	0.3511
Lipids digestibility			
1	61.38	61.80	0.7745
2	66.16	65.78	0.7952
3	69.19	68.74	0.7506
NDF digestibility			
1	54.73	54.82	0.9248
2	45.68	47.38	0.057
3	52.01	53.84	0.04
Cellulose digestibility			
1	53.26	56.07	0.0699
2	51.58	55.97	0.005
3	54.01	59.56	0.0004
Starch digestibility			
1	96.65	97.03	0.0059
2	95.46	97.09	<0.0001
3	95.03	97.06	<0.0001
Ash digestibility			
1	65.99	65.95	0.9637
2	64.27	61.93	0.01
3	68.31	64.96	0.0005

[1]sampling period 1: day 20-21; [2]sampling period 2: day 100-101; [3]sampling period 3: day 180-181.

el, might lead to impaired assimilation of nutrients, to microbial dysbiosis, as well as to an enhanced local and systemic inflammation and immune reaction, caused also by the transmigration of toxins and antigens from the lumen of the hindgut to the blood stream. Those aspects have negative drawbacks on animal health, leading to some systemic issues, usually associated with ruminal acidosis [18,20]. Moreover, energy availability for production purposes is reduced in those conditions [18-20].

High-starch diets, such as the ones often used in confined beef cattle farming, can lead to alteration even of the hindgut environment. In those diets, the flow of starch to the hindgut can be higher, leading to a more pronounced fermentation of it in that location, thus reducing its pH. Both the hindgut microflora and epithelium can be negatively affected by those conditions, even more then the rumen, due to anatomical, microbial, and functional differences [19,21].

The administration of protected forms of gluconate has been reported to positively affect production performance in dairy cows [28-31].

Considering the results of the present trial, the increased growth performance and the better production efficiency recorded in the Treatment group are in line with the main findings recorded in dairy cows [29,30]. Both an improvement of the fermentations, with higher VFA production, at the hindgut level, as well as a more functional and intact epithelial barrier, with a lower stimulation of the proinflammatory status and lower passage of toxic compounds to the blood stream, can be considered as the main reasons of the better performance highlighted in the present trial, in agreement with the main bibliographical findings [22,28-31].

Indeed, in the present condition, treated animals showed a significantly higher digestibility of starch and NDF. Even if the hindgut is reported to contribute less than the rumen to VFA production, about 4% to 5% of the starch and 5% to 12% of the NDF digestions are reported to happen at the hindgut level [18,33,37]. Thus, even if the higher improvements might be seen in terms of digestibility when targeting the rumen, some achievements can be also seen if the hindgut microflora is preserved and stimulated correctly. In this light, the administration of gluconate might have led to a less acidotic, by converting the lactic acid into butyrate, and more stable environment in the hindgut, that is functional and predispose to a higher vitality and efficiency of the gut microbiota, also reducing the risk of dysbiosis at that level [29,30,36]. Indeed, strong reduction of the pH and conditions of hindgut acidosis are often correlated with a reduction in the total tract digestibility of different nutrients [35].

Moreover, the better digestibility found in the present study, is also in line with the findings of Koyun et al. (2022), that recorded and increased VFA concentrations in hindgut digesta of growing steers fed with protected forms of gluconate [32]. Moreover, in several studies the milk fat composition of dairy cows treated with protected forms of gluconate, was different compared to the control ones, showing the different fermentation patterns and fatty acids production in the hindgut [28-30].

Besides the microflora, the more stable and less acidic condi-

Table 6 - Health issues recorded during the fattening period in the two study groups.

Parameter, % (n[1])	Control	Treatment	P Value
BRD[2] morbidity, % (n)	10.00 (10)	7.92 (8)	0.2585
Lameness[3], % (n)	7.00 (7)	0.99 (1)	0.0282
Enterotoxiemy, % (n)	2.00 (2)	0.00 (0)	0.246
Moved to infirmary pen, % (n)	2.00 (2)	0.99 (1)	0.612
Mortality, % (n)	2.00 (2)	0.99 (1)	0.6212

[1]n=number of animals; [2]BRD= bovine respiratory disease; [3]Lameness = all the cases recorded as "lameness" were derived from nutritional issues

tions are also favourable for the intestinal epithelium. More acidotic conditions can indeed lead to a higher degree and easiness of damage to the intestinal epithelium, that is even more sensible compared to the ruminal one, due to their substantial anatomical differences, as well as to the lower buffering potential.

Those damages can negatively alter the absorption potential of the intestine, beside leading to all the other health and production issues related to the proinflammatory status and to the passage of toxins from the lumen of the intestine to the blood stream, caused by the damaged gut epithelium [20]. Moreover, the core function of protected gluconate is to stimulate the production of butyrate, that is recognized as one of the main energy and nutrient sources for the epithelial cells. Likely, the treatment can improve and fortify the epithelial barrier in the hindgut, limiting thus the onset of a proinflammatory status and the transmigration of toxins, such as LPS, to the blood stream, caused by the "leaky gut" phenomenon. Thus, the systemic health consequences, such as laminitis and enterotoxaemia, can be further modulated by protected forms of gluconate as a precursor of butyrate. This aspect is also confirmed in the present trial since the incidence of lameness with nutritional origin was significantly lower in treated animals. Since lameness is correlated with both a reduced feed intake and increased utilization of the available energy to counteract the inflammation instead of being used for production purpose, the lower incidence of it can also have contributed to the better production performances [38-40]. Also, even if not statistically significant, two animals in the control group died because of enterotoxaemia while no animals in the treated groups showed signs of this issue. Possibly, a more resilient and more intact gut epithelial barrier in the treated animals might explain these lower sudden deaths.

CONCLUSIONS

In conclusion, the supplementation of protected sources of calcium gluconate in beef cattle diet can effectively become a strategic approach to improve both production performance and sustainability in beef cattle farming.

Both growth performance, production efficiency and animal health were improved as a result of a healthier and more functional and resistant hindgut in treated animals. Both the hindgut microflora and the integrity of the gut epithelial barrier can be effectively improved and stimulated by the administration of protected sources of calcium gluconate, stimulating thus the digestion and absorption of the nutrients, as well as the overall animal health.

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SUPPLEMENTARY TABLES

Supplementary Table 1 - Chemical composition of the two different TMR in the different phases as recorded by the near-infrared system (Polispec). Besides humidity and dry matter, the data are expressed in percentage on dry matter basis.

Phase[1]	Group	Humidity	Dry matter	Ash	Crude protein	Fats	NDF[2]	ADF[3]	Cellulose	Hemicellulose	ADL[4]	AIA[5]	Starch	Sugars
Phase 1	Control	35.89	64.12	6.32	13.40	2.99	30.14	19.22	16.51	10.93	2.71	0.83	41.68	5.48
	Treatment	36.75	63.25	6.44	13.36	3.05	29.94	19.93	17.23	10.02	2.70	0.81	41.74	5.48
Phase 2	Control	42.56	56.48	6.19	13.20	3.31	25.47	18.79	16.11	6.68	2.68	0.60	45.95	5.88
	Treatment	41.94	56.65	6.16	13.14	3.38	25.18	18.59	15.93	6.59	2.66	0.66	46.31	5.83
Phase 3	Control	35.89	64.12	6.38	12.84	3.01	26.11	17.22	14.56	8.89	2.66	0.63	46.01	5.65
	Treatment	36.75	63.25	6.28	12.79	3.02	25.79	17.93	15.29	7.87	2.64	0.66	46.61	5.52

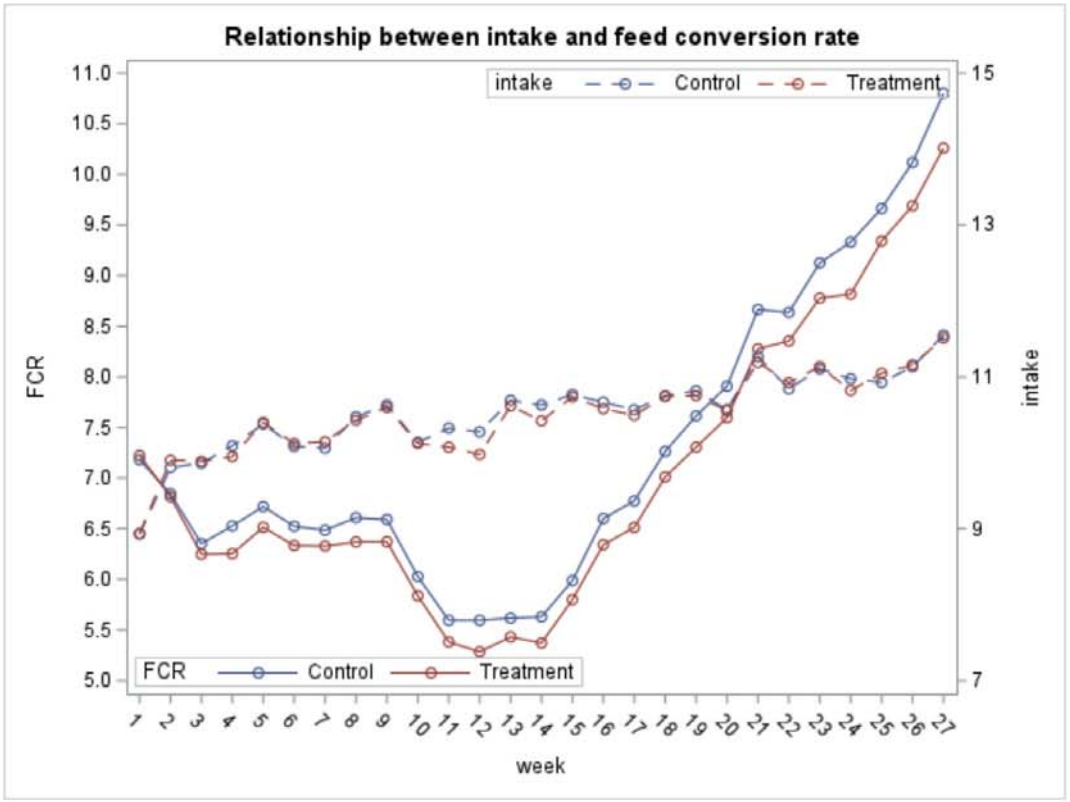
[1]Phase 1= day 20-21; Phase 2= day 100-101; Phase 3= day 180-181; [2]NDF= neutral detergent fiber; [3]ADF= acid detergent fiber; [4]ADL= acid detergent lignin; [5]AIA= acid insoluble ash

Supplementary Table 2 - Chemical composition of the faeces of the two groups in the different phases as recorded by the near-infrared system (Polispec). Besides humidity and dry matter, the data are expressed in percentage on dry matter basis.

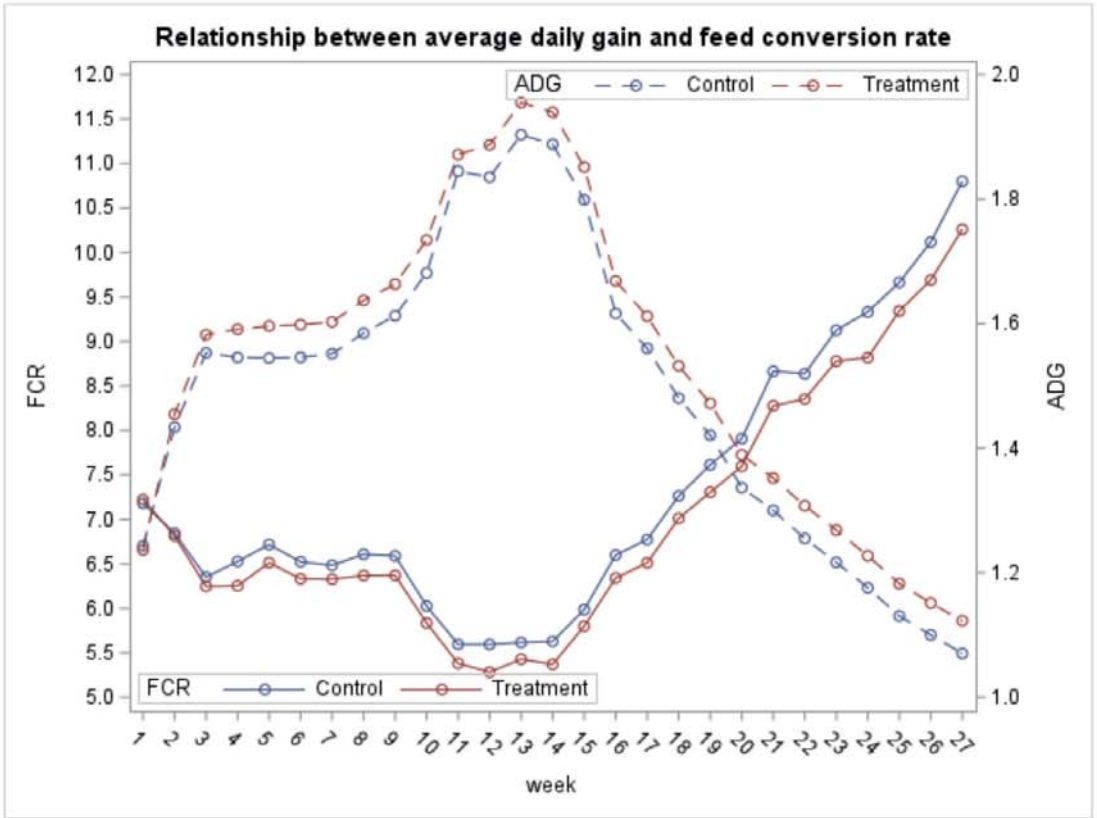
Phase[1]	Group	Humidity	Dry matter	Ash	Crude protein	Fats	NDF[2]	ADF[3]	Cellulose	Hemicellulose	ADL[4]	AIA[5]	Starch	Sugars
Phase 1	Control	82.04	17.97	10.04	13.73	5.40	63.92	50.12	37.40	12.71	13.80	1.46	6.54	0.37
	Treatment	82.23	17.77	10.37	13.81	5.50	64.06	49.60	36.79	12.81	12.93	1.37	5.89	0.38
Phase 2	Control	82.53	17.49	9.88	13.52	5.00	61.82	50.11	38.12	11.99	11.71	1.33	9.33	0.47
	Treatment	80.88	19.13	10.03	16.02	5.31	61.94	47.38	35.06	12.32	13.04	1.27	6.23	0.48
Phase 3	Control	82.44	17.56	9.85	13.02	4.51	61.06	45.44	32.42	13.03	15.62	1.40	11.17	0.40
	Treatment	82.23	17.77	11.24	15.38	4.80	61.10	43.65	30.08	13.57	16.19	1.34	7.03	0.46

[1]Phase 1= day 20-21; Phase 2= day 100-101; Phase 3= day 180-181; [2]NDF= neutral detergent fiber; [3]ADF= acid detergent fiber; [4]ADL= acid detergent lignin; [5]AIA= acid insoluble ash

SUPPLEMENTARY FIGURES



Supplementary Figure 1 - Relationship between weekly feed intake (FI) and weekly feed conversion rate (FCR) in the "Beefmonitor population".



Supplementary Figure 2 - Relationship between weekly average daily gain (ADG) and weekly feed conversion rate (FCR) in the "Beefmonitor population".