Animal 17 (2023) 100791

Contents lists available at ScienceDirect

Animal

The international journal of animal biosciences

Evaluation of tributyrin supplementation in milk replacer on diarrhoea occurrence in preweaning Holstein calves

Matteo Dell'Anno *, Elena Scaglia, Serena Reggi, Silvia Grossi, Carlo Angelo Sgoifo Rossi, Sara Frazzini, Valentina Caprarulo, Luciana Rossi

Department of Veterinary Medicine and Animal Sciences – DIVAS, Università degli Studi di Milano, 26900 Lodi, Italy

A R T I C L E   I N F O

Article history:
Received 29 November 2022
Revised 14 March 2023
Accepted 21 March 2023
Available online 30 March 2023

Keywords:
Antibiotics reduction
Feed additives
Functional nutrition
Neonatal calf diarrhoea
Short-chain fatty acids

A B S T R A C T

Neonatal calf diarrhoea is one of the most important health challenges in cattle herds causing substantial economic losses and antimicrobial use. Due to the raising problem of antimicrobial resistance, effective alternatives are urgently required, in line with European policies. The aim of this study was to evaluate the effect of tributyrin supplementation in milk replacer on diarrhoea, performance and metabolic status in preweaning Holstein calves. Twelve newborn calves, after colostrum administration, were randomly allotted in two experimental groups for 42 days: control (**CTRL**) fed milk replacer, tributyrin (**TRIB**) fed milk replacer supplemented with 0.3% of liquid tributyrin on milk powder weight. Calves BW was recorded on a weekly basis from day 7 to day 42, and feed intake was recorded daily to calculate zootechnical performance. Faecal consistency was assessed daily through the faecal score (0–3 scale; considering diarrhoea moderate = 2 and severe = 3). Faecal samples were collected weekly from rectal ampulla for microbiological analysis by plate counting method evaluating the number of total bacteria, lactic acid bacteria and coliform bacteria. On day 0 and day 42, individual blood samples were collected from jugular vein for metabolic profile analysis. Serum samples of day 42 were also evaluated for the antioxidant barrier using a colorimetric test, while glucagon-like peptide 2 and diamine oxidase concentrations were measured through immunoenzymatic assays. Tributyrin supplementation did not influence the zootechnical performance of calves over 42 days of trial. Diarrhoea frequency was significantly lower in TRIB compared to CTRL group (27.91 and 38.37%; $P < 0.01$) considering the whole experimental period. In particular, the major effect was observed for moderate diarrhoea in TRIB group that showed a significantly reduced frequency compared to CTRL ($P < 0.01$) thus suggesting a preventive effect of tributyrin. Faecal total bacterial, lactic acid and coliform bacteria counts did not show differences between groups. Urea serum concentrations tended to be lower in TRIB compared to CTRL, indicating an efficient utilisation of dietary protein. Antioxidant barrier and glucagon-like peptide 2 were comparable between CTRL and TRIB on day 42. Diamine oxidase concentrations were significantly decreased in TRIB compared to CTRL group after 42 days of trial ($P < 0.01$), suggesting a higher gut epithelial integrity probably due to lower diarrhoea frequency and the nourish effect of tributyrin on enterocytes. In conclusion, tributyrin could be considered as a valuable bioactive feed additive to decrease the neonatal diarrhoea occurrence and support intestinal integrity in preweaning calves.

Implications

Neonatal calf diarrhoea significantly impacts the farms' profitability. Antibiotic treatments are used to limit the detrimental effects of gastrointestinal infections. The global issue of antimicrobial resistance prompted all international organisations to limit their use and encouraged the research for sustainable alternatives to improve animal health and decrease pathologies incidence. In animal farming, alternatives to antibiotics are defined as any compound that may decrease the antibiotics use. In this study, the effect of tributyrin supplementation in milk replacer was evaluated in preweaning calves. Tributyrin supplementation at 0.3% significantly decreased the diarrhoea frequency and improved the intestinal integrity after 42 days. Even if antibiotic treatments cannot be fully substituted, tributyrin could be considered an interesting bioactive feed additive to prevent

* Corresponding author at: Department of Veterinary Medicine and Animal Science, via dell'Universtià 6, 26900 Lodi, Italy.

E-mail addresses: matteo.dellanno@unimi.it (M. Dell'Anno), elena.scaglia@unibs.it (E. Scaglia), serena.reggi@unimi.it (S. Reggi), silvia.grossi@unimi.it (S. Grossi), carlo.sgoifo@unimi.it (C.A. Sgoifo Rossi), sara.frazzini@unimi.it (S. Frazzini), valentina.caprarulo@unibs.it (V. Caprarulo), luciana.rossi@unimi.it (L. Rossi).

https://doi.org/10.1016/j.animal.2023.100791
1751-7311/

diarrhoea and decrease antibiotic treatment in calves during the preweaning phase.

Introduction

Neonatal calf diarrhoea (**NCD**) is a well-known and globally diffused disease in dairy farms, which negatively impacts profitability and animal welfare. NCD can be responsible for 75% of calf mortality during the preweaning period (Khawaskar et al., 2021). Moreover, NCD is also related to indirect losses due to higher morbidity, increased needs and costs of treatments and growth retardation, also with negative effects on further dairy productive life and productivity (e.g. delayed first calving) (Brunauer et al., 2021). NCD is a multifactorial disease where infectious agents, (e.g., Rotavirus, *Escherichia coli* and *Salmonella* spp.), animal (age, immunity, general health status) and environmental-related factors (colostral consumption, calf housing, hygienic conditions) can concur to its onset and incidence (Cho and Yoon, 2014; Windeyer et al., 2014).

To limit the incidence and detrimental effects of NCD, antibiotics are needed to treat calves when watery diarrhoea occurs (Eibl et al., 2021). Antimicrobial resistance is an important multifaceted issue on Earth, a transversal concern for several animal species (Rossi et al., 2021), that requires to be coped with an integrated approach in line with One Health principles. Besides the ban of antibiotics as growth promoters in animal feeds (European Parliament Regulation EC 1831/2003), the persistent emergence of antimicrobial resistance prompted the European Union to introduce more limitations for veterinary drug use (Reg. EU 6/2019; WHO, 2019; EMA, 2020).

Thus, alternative strategies to reduce the incidence of NCD in calves are required. Alternatives to antibiotics have been defined as any compound able to reduce the antibiotic use. In the light of this, functional nutrition can become a vehicle for bioactive molecules, which can sustain animal health by enhancing the calf's immune system, gastrointestinal development and functionality, thus reducing the risk of pathology development (Grossi et al., 2021).

Butyrate is a short-chain fatty acid, also produced by bacteria in the gut, which nourishes enterocytes for their development and proliferation. In addition, butyrate exerts an antimicrobial effect against a wide range of Gram+ and Gram-bacterial strains (Namkung et al., 2011; Kovanda et al., 2019). It has been demonstrated that dietary supplementation of butyrate salts can increase growth performance, stimulate gastrointestinal development, and enhance nutrient digestion in calves (Guilloteau et al., 2009; Górka et al., 2018; Liu et al., 2021b). However, providing butyrate salts can be challenging due to their short half-life in blood plasma and unpleasant smell that may affect the feed palatability.

Tributyrin(butanoic acid 1,2,3-propanetriyl ester) is a flavouring feed additive composed of three butyric acid molecules, that can be used as an alternative to overcome these challenges. In this form, it has more favourable pharmacokinetics properties and does not have the butyrate-distinctive odour.

Tributyrin dietary supplementation has been reported to enhance growth performance, intestinal morphology, digestive and barrier function in weaned piglets (Hou et al., 2014; Dong et al., 2016; Sotira et al., 2020). A recent study showed that tributyrin supplementation may shift the gut microbiota population in weaned piglets by increasing beta diversity and relative abundance of *Desulfovibrio* spp., *Mucipirillum* spp., *Butyrivibio* spp. and *Oscillobacter* spp. (Miragoli et al., 2021).

The effect of tributyrin supplementation in calves has been investigated by a limited number of studies with contrasting findings. Tributyrin is mainly released in the gut, possibly preventing NCD and improving digestion efficiency in preweaned calves fed milk replacers. Increased animal performance has been observed with tributyrin supplementation in milk replacer in preweaning calves (Liu et al., 2022). On the contrary, Araujo et al. (2015) and Inabu et al. (2019) reported a reduction of zootechnical performance by supplementing tributyrin in calves.

However, the effect of tributyrin supplementation on diarrhoea prevention has not been investigated yet. The aim of this study was to assess the tributyrin supplementation in milk replacer as feed additive on diarrhoea occurrence, performance and metabolic status in preweaning Holstein calves.

Material and methods

Animal housing and experimental design

The experimental trial was approved by the Animal Welfare Organisation of University of Milan (OPBA authorisation n° 79/2020) and performed in accordance with European regulations.

Within 4 hours from birth, twelve Holstein calves were fed with 4 L of high-quality colostrum by bottle feeding with two portions and housed in individual straw-bedded pens with free access to water under homogeneous environmental conditions for 42 days. After 24 hours, animals were allotted in two groups, balanced per weight and sex: control group (**CTRL**) fed milk replacer (complementary feed) composed by: skimmed milk, milk whey powder, palm oil, coconut oil, sunflower oil, milk whey lactose and mineral-free, serum whey powder without lactose, wheat flour gelatinised, dextrose (Lattover, Veronesi Verona S.p.A., Verona, Italy), and tributyrin group (TRIB) fed milk replacer supplemented with 0.3% of liquid tributyrin (feed additive approved by the Reg. EC 1831/2003, code 2b09211; New Feed Team Srl, Lodi, Italy) on milk replacer powder. Dietary treatments were provided after colostrum assumption, animals fed twice per day increasing the amount of milk replacer, following routine farm practices (Table 1). Calves were fed twice per day starting from 4 L of reconstituted milk replacer (100 g of powder/L of water according to labelling information) and progressively increasing the amount of milk replacer following the animals' growth curve (Amaral-Phillips et al., 2006). Calves with clinical signs of severe diarrhoea (faecal score = 2) were re-hydrated with a commercial product formulated with protopectins, algae and yeasts for supporting intestinal health during gastrointestinal disorders (Omindiar®; MSD Animal Health S.r.l., Segrate, Milan, Italy).

Zootechnical performance, diarrhoea frequency and sample collection

Calves BW was individually recorded weekly from day 7 to day 42. The average daily gain (ADG) was calculated by dividing the weekly weight gain for the time period. Consumed milk was registered daily for the calculation of average daily feed intake (ADFI). The feed conversion ratio (FCR) was evaluated by dividing the ADG and ADFI on a weekly basis. Faecal consistency was scored on a daily basis for each animal using a four-point scale (0 = dry; 1 = normal; 2 = runny, considered moderate diarrhoea; 3 = watery, considered severe diarrhoea) defining a score > 1 indicative of diarrhoea (Santos et al., 2015; Gomez et al., 2017). The frequency of diarrhoea was evaluated as the percentage of observations of animals with clinical signs of diarrhoea divided by the total observation performed over the considered period (Diarrhoea frequency = n° of faeces with score 2 and/or 3 divided by the total number of observations for the considered period). Faecal samples were collected from rectal ampulla weekly for microbiological analysis.

Table 1
Nutritional composition of milk replacer in the Holstein calf trial.

Analyte	Composition (% as fed)[1]
CP	23.00
EE	18.00
CF	0.10
Ashes	7.50
Lys	2.10
Ca	1.00
P	0.70
Na	0.50

Milk replacer (Lattover, Veronesi Verona S.p.A., Verona, Italy) composition: skimmed milk, milk whey powder, palm oil, coconut oil, sunflower oil, lactose and mineral-free milk whey, lactose-free serum whey powder, gelatinised wheat flour, dextrose. The milk was prepared using 100 g of product for 1 L of water.
Additives per Kg: Vitamins, pro-vitamins and substances with similar effect = Vitamin A 20 000 IU, Vitamin D3 4 000 IU, Vitamin E 100 mg, Vitamin C 150 mg, Vitamin B1 6 mg, Vitamin B2 12 mg, Vitamin B6 6 mg, Vitamin B12 80 mg, Niacin 30 mg, Calcium D-pantothenate 25 mg, Vitamin K3 4 mg, Betain hydrochloride 250 mg; Trace elements = Iron 75 mg, Copper 6 mg, Zinc 85 mg, Iodine 1 mg, Manganese 30 mg, Selenium 0.3 mg. Abbreviations: EE = ether extract; CF = crude fibre; Lys = lysine; Ca = calcium; P = phosphorous; Na = sodium.
[1] Percentages of nutrients declared by the producer (Veronesi Verona S.p.A., Verona, Italy).

On day 0 and day 42, blood samples were collected from jugular vein using vacuum tubes without anticoagulants for serum metabolites profile, antioxidant barrier, and immunoenzymatic analyses.

Nutrient composition of the milk replacer

The milk replacer used in this study was analysed in triplicate in terms of principal nutrients (AOAC, 2019): DM, CP, ether extract (**EE**), ash content. DM was obtained by drying milk powder in a forced air oven at 65 °C for 24 h (AOAC method 930.15). CP was determined by the Kjeldahl method (AOAC method 2001.11). EE was determined after acid hydrolysis (3 N HCl) using ether extraction with a Soxtec system (AOAC method 2003.05). Ash content was obtained by incinerating samples in a muffle furnace at 550 °C (AOAC method 942.05). Each determination was performed in triplicate.

Microbiological analysis of faecal samples

The faecal samples were analysed by plate counting method using three different culture media: Plate Count Agar (**PCA**) for total bacteria, De Man, Rogosa and Sharpe Agar (**MRS**) for lactic acid bacteria and Violet Red Bile Lactose Agar for coliform bacteria. Briefly, 1 g of each faecal sample was diluted in 9 mL of sterile 0.9% NaCl and centrifuged (3 000 rpm for 10 min) to collect the supernatant. Samples were serially diluted, and bacteria were enumerated by plate counting after 24 h of semi-anaerobic incubation at 37 °C using the overlay method for MRS and VRBA, and the inclusion method for PCA (Dell'Anno et al., 2021). The lactic acid:coliform bacteria ratio was calculated based on plate counting results which were expressed as $\log_{10}$ of colony-forming units per gram of dried faeces ($\log_{10}$CFU/g). Each sample was analysed in triplicate.

Metabolic profile, antioxidant barrier and immunoenzymatic evaluation of blood samples

Blood serum was obtained by centrifugation at 3 000 rpm for 15 minutes from blood samples collected at 0 and 42 days. Serum was analysed to determine the enzymatic activity or concentration of alanine aminotransferase (ALT-GPT; IU/L), albumin (g/L), albumin/globulin (A/G ratio), creatinine (μmol/L), globulin (g/L), glucose (mmol/L), high-density lipoprotein (HDL; mmol/L), low-density lipoprotein (LDL; mmol/L), magnesium (mmol/L), phosphorus (mmol/L), total bilirubin (μmol/L), total cholesterol (mmol/L), total protein (g/L), triglycerides (mmol/L), and urea (mmol/L) using a multiparametric autoanalyzer for clinical chemistry (ILab 650; Instrumentation Laboratory Company, Lexington, MA, USA) according to Dell'Anno et al. (2021).

Serum samples of day 42 were analysed in triplicate through the Oxy-Adsorbent test (Diacron, Grosseto, Italy) to determine the serum antioxidant barrier according to the manufacturer's instruction. Endpoint absorbances were measured after 10 minutes of incubation at 37 °C using a UV–Vis spectrophotometer (V630 UV–Vis, Jasco GmbH, Pfungstadt, Germany) at 546 nm.

Serum Glucagon-Like Peptide 2 (**GLP-2**) and Diamine Oxidase (**DAO**) concentrations were also quantified in duplicate in serum samples of 42 days by using enzyme-linked immunosorbent assay kits specific for bovine samples according to the manufacturer's instructions (Bioassay Technology Laboratory, Shanghai, China). Absorbances were measured with a microplate reader at 450 nm (Bio-Rad 680 microplate reader, Bio-Rad Laboratories, Inc., Hercules, CA, USA), and concentrations were calculated according to the respective standard curves.

Statistical analysis

Obtained data were tested for normal distribution with the Shapiro-Wilk test evaluating the Q-Q plots using JMP Pro 15® (SAS Inst. Inc., Cary, NC, USA). Results of zootechnical performance and plate counting were analysed using a linear model including the fixed effect of treatment (Trt), time (Time), the interaction between treatment and time (Trt × Time), while each animal was included as random factor. Frequencies of faecal score were converted into a dichotomous variable (normal/pathological) considering diarrhoea for a registered faecal score > 1, moderate diarrhoea frequency was calculated for each faecal score = 2 and severe for a faecal score = 3. The observed frequencies were assessed using the chi-squared test, allowing to evaluate if differences between observed and expected frequencies were caused by treatments or only by casualty. Serum metabolites data were evaluated after performing the ANCOVA to adjust the initial variability of serum samples. GLP-2, DAO and Oxy-Adsorbent values of 42 days were analysed using the Student's t-test for unpaired groups. Pairwise comparisons were evaluated by using Tukey's Honestly Significant Difference test (Tukey's HSD). Results were presented as least squares means ± SE. Means or frequencies were considered statistically different when $P \leq 0.05$.

Results

Nutrient composition of the milk replacer, zootechnical performance, and diarrhoea occurrence

Milk replacer composition analysis showed results in line with product labelling (Table 2). Calves showed no differences in zootechnical performance over the 42 days of trial (Table 3). From

Table 2
Analysed nutrient composition of milk replacer in the Holstein calves trial.

Analyte	Composition (% as fed)
DM	98.45 ± 0.05
CP	21.16 ± 0.72
EE	17.52 ± 1.33
Ashes	6.61 ± 0.07

Data are presented as means ± SD.

7 to 42 days, the BW, ADG and ADFI progressively increased without highlighting significant differences between experimental groups.

The diarrhoea occurrence showed a significantly higher diarrhoea frequency in CTRL group compared to TRIB group over the 42 days of experimental trial (38.37% for CTRL and 27.91% for TRIB group; $P < 0.01$). In particular, CTRL showed a higher frequency of diarrhoea during the period from 14 to 21 and 28 to 35 days compared to TRIB group ($P < 0.01$; Table 4). Specifically, the frequency of moderate diarrhoea cases was significantly lower in TRIB compared to CTRL group over 42 days of trial, according to the faecal consistency score ($P < 0.01$; Fig. 1). The frequency of severe diarrhoea showed a significant decrease in TRIB compared to CTRL only for the period from 28 to 35 days (7.14 and 0.00%, $P < 0.01$).

Faecal bacteria plate counting

Plate counting of faecal samples did not show significant differences for coliform bacteria and lactic acid:coliform bacteria ratio (Table 5). Even if a significant effect of the interaction (Trt × Time) was registered for the total bacterial count and lactic acid bacteria, pairwise comparisons did not highlight any statistically significant difference. The total bacterial count of CTRL group tended to be increased at 7 days of trial compared to TRIB group ($P = 0.088$).

Serum metabolic profile, antioxidant barrier level, glucagon-like peptide 2 and diamine oxidase concentrations

Serum metabolic profile displayed no significant differences between CTRL and TRIB groups for all the considered parameters (Table 6). Only, the urea level in TRIB group tended to be lower compared to CTRL at 42 days of trial ($P = 0.058$).

The serum antioxidant barrier, evaluated through Oxy-Adsorbent test, did not show significant differences between CTRL and TRIB group after 42 days (191.10 ± 8.66 µmol HClO/mL and 211.22 ± 8.66 µmol HClO/mL, respectively). Glucagon-like peptide 2 did not reveal statistical differences between experimental groups on day 42 of trial (97.25 ± 3.36 ng/L for CTRL and 100.12 ± 3.36 ng/L for TRIB group). Diamine oxidase hematic values were significantly lower in TRIB compared to CTRL group after 42 days (Fig. 2; $P < 0.01$).

Table 4
Diarrhoea frequency divided per diarrhoea occurrence (faecal score > 1), moderate (faecal score = 2) and severe (faecal score = 3) of calves in the control (CTRL) and tributyrin (TRIB) groups registered during the 42 days of trial.

Item	CTRL	TRIB	*P*-value
Frequency of diarrhoea			
d 0–7	34.52%	36.90%	0.6289
d 7–14	29.76%	28.57%	0.8248
d 14–21	14.29%[a]	5.95%[b]	0.0543
d 21–28	8.33%	2.38%	0.0703
d 28–35	13.10%[a]	2.38%[b]	0.0047
d 35–42	15.63%	8.33%	0.0921
Moderate diarrhoea			
d 0–7	21.43%	15.48%	0.2575
d 7–14	25.00%	19.05%	0.2712
d 14–21	13.10%	5.95%	0.0922
d 21–28	8.33%	2.38%	0.0703
d 28–35	5.95%	2.38%	0.2293
d 35–42	8.33%	6.25%	0.5625
Severe diarrhoea			
d 0–7	13.10%	21.43%	0.1069
d 7–14	4.76%	9.52%	0.2085
d 14–21	1.19%	0.00%	0.2370
d 21–28	0.00%	0.00%	–
d 28–35	7.14%[a]	0.00%[b]	0.0030
d 35–42	7.29%	2.08%	0.0723

[a,b] Different lowercase letters indicate statistically significant differences between groups. Animals in the control group (CTRL) fed milk replacer; treatment group fed milk replacer supplemented with 0.3% of liquid tributyrin (TRIB) (ACIFIS® Tri-B, New Feed Team, Lodi, Italy).

Table 3
Zootechnical performance in the experimental Holstein calves trial (from day 7 to 42) for the control (CTRL) and treatment (TRIB) groups.

			P-values		
Item	CTRL	TRIB	Trt	Time	Trt × Time
BW, kg			0.8018	<0.0001	0.9690
Birth weight	38.73 ± 2.61	38.12 ± 2.61			
d 7	37.57 ± 2.59	36.47 ± 2.59			
d 14	38.87 ± 2.59	38.42 ± 2.59			
d 21	42.02 ± 2.59	40.53 ± 2.59			
d 28	45.03 ± 2.59	44.18 ± 2.59			
d 35	48.88 ± 2.59	47.93 ± 2.59			
d 42	52.48 ± 2.59	51.78 ± 2.59			
ADFI, g/day			0.6534	<0.0001	0.9295
d 7–14	485.71 ± 24.99	492.86 ± 24.99			
d 14–21	529.76 ± 24.99	557.14 ± 24.99			
d 21–28	580.95 ± 24.99	585.72 ± 24.99			
d 28–35	639.29 ± 24.99	661.90 ± 24.99			
d 35–42	440.48 ± 24.99	440.48 ± 24.99			
ADG, g/day			0.4496	0.4162	0.8868
d 7–14	346.26 ± 95.60	464.08 ± 95.56			
d 14–21	450.00 ± 76.84	422.77 ± 84.83			
d 21–28	430.95 ± 76.84	521.43 ± 76.84			
d 28–35	550.00 ± 76.84	535.72 ± 76.84			
d 35–42	514.29 ± 76.84	550.00 ± 76.84			
FCR, g/g			0.9018	0.7193	0.4531
d 7–14	1.07 ± 0.46	1.06 ± 0.51			
d 14–21	1.15 ± 0.46	0.98 ± 0.46			
d 21–28	1.04 ± 0.46	2.17 ± 0.46			
d 28–35	1.50 ± 0.46	1.11 ± 0.46			
d 35–42	1.64 ± 0.46	1.27 ± 0.46			

Data are presented as least squares means ± SE. Animals in the control group (CTRL) fed milk replacer; treatment group fed milk replacer supplemented with 0.3% of liquid tributyrin (TRIB) (ACIFIS® Tri-B, New Feed Team, Lodi, Italy). Abbreviations: ADFI = average daily feed intake of milk replacer powder; ADG = average daily gain; FCR = feed conversion ratio.

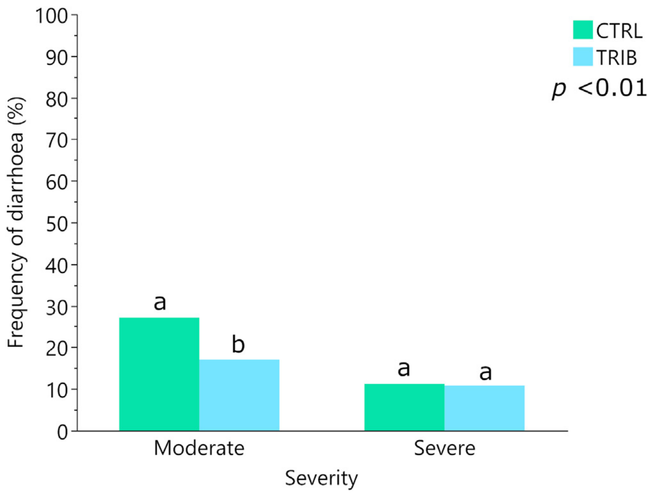

Fig. 1. Frequency of moderate and severe diarrhoea cases registered on total observations during the 42 days of trial for control (CTRL) and tributyrin (TRIB) group. Frequencies are presented as the percentage of observation of animals with clinical signs of diarrhoea on total observation performed over 42 days. Moderate diarrhoea was defined as faecal score = 2 and severe diarrhoea was defined as faecal score = 3. [a,b] Different lowercase letters indicate statistically significant differences between groups (P < 0.01). The trial of the following study was conducted on Holstein calves (*Bos taurus*). Animals in the control group (CTRL) fed milk replacer; treatment group fed milk replacer supplemented with 0.3% of tributyrin (TRIB) (ACIFIS® Tri-B, New Feed Team, Lodi, Italy).

Discussion

The present study investigated the tributyrin supplementation in milk replacer on zootechnical performance, diarrhoea occurrence, metabolic, antioxidant and intestinal barrier status in preweaning calves.

The inclusion of tributyrin in milk replacer in preweaned calves did not affect the growth performance over 42 days of trial. Accordingly, Inabu et al. (2019) registered no positive effect of tributyrin supplementation in milk replacer on animal growth in preweaning calves. Moreover, Frieten et al. (2017) observed that butyrate sodium salt supplementation in milk replacer did not display a positive effect on ADG in preweaned calves. Newborn calves showed an initial decrease in BW during the first week after birth in both experimental groups. This could be ascribed to the high occurrence of diarrhoea registered from 0 to 7 days after birth (48.33% in CTRL and 36.90% in TRIB of total observations), which exert a well-known negative effect on growth performance (Karamzadeh-Dehaghani et al., 2021). After the first week, calves' growth increased progressively in both groups after 7 days without showing statistical differences. Recent recommendations for calves' nutrition suggest the higher quantity of milk as well as the higher density of milk replacer in comparison with the nutritional scheme adopted in the following protocol. This aspect could have influenced the growth rate, even if it is important to mention that from 0 to 14 days after birth, the highest diarrhoea occurrence was registered, and the presence of gastrointestinal disorders could have affected the weight gain.

Table 5
Microbiological evaluation by plate courting of faecal samples of the control (CTRL) and tributyrin (TRIB) groups over 42 days of Holstein calves trial.

				P-values		
Counted bacteria	CTRL	TRIB	SE	Trt	Time	Trt × Time
Total bacterial count (log_{10}CFU/g of dried faeces)				0.1069	<0.0001	0.0119
d 0	8.65	7.85	0.25			
d 7	8.63*	7.48*	0.25			
d 14	7.43	7.90	0.25			
d 21	7.12	7.38	0.25			
d 28	7.09	7.29	0.25			
d 35	7.37	6.85	0.25			
d 42	7.24	6.93	0.25			
Lactic acid bacteria (log_{10}CFU/g of dried faeces)				0.2364	<0.0001	0.0049
d 0	8.22	7.36	0.26			
d 7	8.02	7.55	0.26			
d 14	7.28	7.61	0.26			
d 21	7.01	7.15	0.26			
d 28	6.63	7.09	0.26			
d 35	7.16	6.40	0.26			
d 42	6.18	7.07	0.26			
Coliform bacteria (log_{10}CFU/g of dried faeces)				0.8515	<0.0001	0.4058
d 0	6.51	6.67	0.37			
d 7	7.02	6.28	0.37			
d 14	5.94	6.48	0.37			
d 21	4.84	5.60	0.37			
d 28	5.65	5.57	0.37			
d 35	5.74	5.59	0.37			
d 42	5.34	5.21	0.37			
Lactic acid:coliform bacteria ratio (log_{10}CFU/log_{10} CFU)				0.5209	0.1756	0.3687
d 0	1.29	1.14	0.09			
d 7	1.16	1.26	0.09			
d 14	1.24	1.21	0.09			
d 21	1.47	1.31	0.09			
d 28	1.18	1.27	0.09			
d 35	1.25	1.16	0.09			
d 42	1.36	1.21	0.09			

Data are presented as least squares means ± SE. Animals in the control group (CTRL) fed milk replacer; treatment group fed milk replacer supplemented with 0.3% of tributyrin (TRIB) (ACIFIS® Tri-B, New Feed Team, Lodi, Italy). Abbreviations: CFU = colony-forming unit.
* Asterisks indicate statistical tendency (P < 0.09).

Table 6
Serum metabolites concentration at 42 days of Holstein calf trial in the control (CTRL) and tributyrin (TRIB) groups.

Serum Metabolite	CTRL	TRIB	SE	*P*-value
Total protein content, g/L	52.55	54.55	1.48	0.3689
Albumin, g/L	30.14	30.19	0.58	0.9568
Globulin, g/L	22.75	23.98	1.27	0.5204
Albumin/Globulin, g/L	1.38	1.33	0.07	0.6493
Urea, mmol/L	2.39*	1.94*	0.14	0.0581
Nonesterified fatty acids (NEFAs), mmol/L	0.26	0.35	0.03	0.1388
Glucose, mmol/L	5.14	5.11	0.37	0.9534
Total cholesterol, mmol/L	3.71	3.86	0.32	0.7497
Triglyceride, mmol/L	0.26	0.31	0.06	0.5526
Aspartate aminotransferase (AST), IU/L	52.28	52.89	2.42	0.8625
Gamma-glutamyl transferase, IU/L	38.75	25.91	10.45	0.4304
Total bilirubin, µmol/L	3.34	2.93	0.47	0.5668
Creatine kinase (CK), IU/L	173.44	139.56	24.90	0.3611
Calcium, mmol/L	2.50	2.57	0.04	0.1886
Phosphorus, mmol/L	2.48	2.52	0.08	0.7664
Magnesium, mmol/L	0.81	0.80	0.03	0.8955
Beta-hydroxybutyrate, mmol/L	0.03	0.02	0.01	0.6103
High-density lipoprotein (HDL), mmol/L	1.80	1.86	0.12	0.7214
Low-density lipoprotein (LDL), mmol/L	1.86	1.93	0.21	0.8235

Data are presented as least squares means ± SE. Animals in the control group (CTRL) fed milk replacer; treatment group fed milk replacer supplemented with 0.3% of liquid tributyrin (TRIB) (ACIFIS® Tri-B, New Feed Team, Lodi, Italy).
* Asterisks indicate statistical tendency ($P < 0.09$).

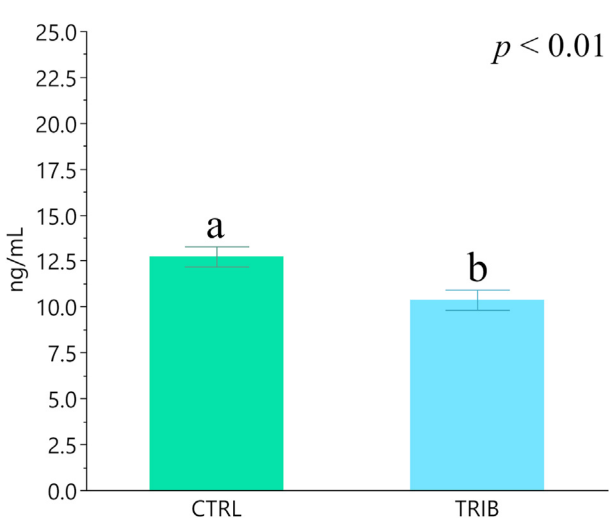

Fig. 2. Diamine oxidase serum levels measured at 42 days of trial for control (CTRL) and tributyrin group (TRIB). Data are expressed as least squares means ± SE. [a,b] Different lowercase letters indicate statistically significant differences between groups ($P < 0.01$). The trial of the following study was conducted on Holstein calves (*Bos taurus*). Animals in the control group (CTRL) fed milk replacer; treatment group fed milk replacer supplemented with 0.3% of tributyrin (TRIB) (ACIFIS® Tri-B, New Feed Team, Lodi, Italy).

The incidence of diarrhoea was significantly lower in TRIB group over 42 days of trial. In particular, CTRL group registered an increased frequency of diarrhoea during the period from 14 to 21 days and 28 to 35 days after birth. The inclusion of functional feed additives with extra-nutritional characteristics has been reported to positively impact animal health and prevent or decrease gastrointestinal disorder occurrence (Gong et al., 2021; Xie et al., 2021). Short-chain fatty acids are widely used as feed additives due to their antibacterial properties (Neath et al., 2022). Among them, butyrate plays a key role in the maintenance of intestinal health and homeostasis. In particular, it has a histone deacetylase inhibitory activity that impacts gene expression by modulating chromatin structures. Several studies demonstrated that butyrate can stimulate the epigenetic regulation for anti-inflammatory and anti-carcinogenic effects (Guilloteau et al., 2010; Berni Canani et al., 2012; Du et al., 2021). In addition, butyrate can modulate the immune response by influencing the metabolism of different cell types such as macrophages (Zhou et al., 2014; Du et al., 2021). The level of 0.3% of tributyrin concentration was selected based on the authorised dose of supplementation in the European Union (European Parliament Regulation EC 1831/2003) and due to the encouraging results observed in previous studies on growth performance, diarrhoea frequency and gut health in preweaning calves (Araujo et al., 2015; Inabu et al., 2019; Liu et al., 2021a). The tributyrin supplementation decreased the moderate diarrhoea occurrence over 42 days of trial, thus suggesting a modulation of intestinal homeostasis through its known effects for microbiota modulation in other species (Miragoli et al., 2021), antimicrobial activity (Hansen et al., 2021) and trophic effect on enterocytes (Leonel et al., 2013). Tributyrin supplementation did not affect the severe diarrhoea cases for more than 80% of the considered period, showing a reduced severe diarrhoea frequency from 28 to 35 days in TRIB compared to CTRL. Obtained findings suggest the limited ability of tributyrin in controlling infections probably suggesting that feed additives with extra-nutritional characteristics cannot fully substitute the therapeutic use of antibiotics in severe cases.

Principal faecal microbial classes revealed no difference in the present trial, suggesting that tributyrin supplementation did not alter the number of total, coliform and lactic acid bacteria. Total bacterial count showed a tendency to decrease in TRIB group on day 7, probably influenced by the initial numerical difference found on day 0 between faecal samples of experimental groups without showing a strong statistical significance.

Nevertheless, the blood metabolic profile showed a tendency to decrease the urea concentration in TRIB compared to CTRL group after 42 days. Urea is a final product of liver synthesis from protein metabolism, and its concentration can be considered as the index of protein balance and feed efficiency (Waguespack et al., 2011). In general, an increased urea level is considered indicative of scarce nitrogen utilisation, and poor protein digestibility (Terré et al., 2021). Besides influencing the intestinal development, butyrate supplemented through the milk replacer can also improve pancreatic functionality, with greater enzyme secretions and thus a more efficient use of feed nutrients, especially amino acids (Hill et al., 2007; Górka et al., 2018). Accordingly, Hill et al. (2007) reported a lower level of urea in preweaned calves supplemented with butyrate. In addition, Sotira et al. (2020) observed a decreased serum urea concentration in pigs by dietary supplementation of tributyrin for 40 days, suggesting a more efficient protein utilisation. It is known that the feed intake affects the blood urea nitrogen concentration, and a reduced energy availability due to low DM intake leads to the mobilisation of protein stores thus increasing the blood urea concentration (Crawford et al., 2022). Considering the growth rate observed in this study and the milk consumption during the first period, even if serum urea showed only a tendency to increase, this aspect could have contributed to the registered increase in blood urea in CTRL group.

Serum GLP-2 and Oxy-Adsorbent test data revealed no differences between experimental groups after 42 days of trial. GLP-2 is a 33 amino acids peptide produced from intestinal L cells after feed ingestion, that plays an important role in the gut morphology and functionality (Khan et al., 2017; Hatew et al., 2019) and its blood level can be a useful indicator of intestinal development (Mutanen and Pakarinen, 2017; Pyo et al., 2020). Oxy-Adsorbent test measures the blood antioxidant barrier as the capacity of serum samples to counter the oxidative stress induced by HClO. These results suggest that tributyrin supplementation did not impair the intestinal morphology and serum antioxidant barrier, suggesting also the possible good health status of animals at the end of the trial.

DAO concentration in blood serum was significantly lower in TRIB group after 42 days of tributyrin supplementation compared to CTRL group. DAO is an anti-histamine enzyme produced by enterocytes and secreted into the lumen during the digestion process (Fukuda et al., 2019). Serum DAO concentration can be considered an indirect biomarker of mucosal integrity (Li et al., 2018). Indeed, higher DAO concentration in blood flow was reported by other authors as associated with mucosal damage (Cai et al., 2019; Ren et al., 2022; Zhang et al., 2022). Cresci et al. (2017) demonstrated the positive impact of tributyrin supplementation to reduce ethanol-induced damage supporting intestinal barrier healing from injury. Specifically, the inclusion of butyrate in milk replacer showed to modulate the gut microbiota, increase mitosis and decrease apoptotic index in the duodenum and jejunum resulting in both a thicker tunica mucosa and longer intestinal villi (Guilloteau et al., 2009; Górka et al., 2018; O'Hara et al., 2018). The observed decreased concentration of DAO could be ascribed to the positive effect of butyrate on gut epithelium integrity, meant as reduced index of microscopic lesions and histological modification of the intestinal barrier (Liu et al., 2012; Fukudome et al., 2014), resulting in a lower diarrhoea occurrence in TRIB group.

Conclusions

Dietary supplementation of 0.3% of tributyrin in milk replacer to Holstein calves for 42 days during the preweaning period resulted in a lower diarrhoea occurrence by reducing the frequency of moderate episodes. Tributyrin tended to lower urea serum concentration, indicating a more efficient protein utilisation, and significantly lowered the diamine oxidase concentration in blood serum, suggesting a higher gut epithelial integrity of supplemented group compared to control after 42 days.

This study demonstrated the potential use of tributyrin as a bioactive feed additive to prevent neonatal calf's diarrhoea and probably supporting the intestinal integrity. More studies will be required to investigate the effect of tributyrin supplementation on gut microbiota in preweaning calves.

Ethics approval

This study was conducted following the guidelines of the Declaration of Helsinki. The experimental trial was approved by the Animal Welfare Organisation of University of Milan (OPBA authorisation number 79/2020).

Data and model availability statement

The data presented in this study are not deposited in an official repository. Data are available within the article and from the corresponding author upon reasonable request.

Authors ORCIDs

M. Dell'Anno: https://orcid.org/0000-0002-2171-864X.
E. Scaglia: https://orcid.org/0000-0002-4225-5234.
S. Reggi: https://orcid.org/0000-0002-2136-1177.
S. Grossi: https://orcid.org/0000-0003-4588-0171.
C.A. Sgoifo Rossi: https://orcid.org/0000-0002-3602-9660.
S. Frazzini: https://orcid.org/0000-0001-8810-0003.
Valentina Caprarulo: https://orcid.org/0000-0003-3691-4608.
L. Rossi: https://orcid.org/0000-0003-1178-4683.

Authors' contributions

Conceptualisation: **Luciana Rossi**; methodology: **Matteo Dell'Anno, Serena Reggi, Elena Scaglia**; investigation: **Matteo Dell'Anno, Serena Reggi, Elena Scaglia, Valentina Caprarulo**; data curation: **Matteo Dell'Anno, Elena Scaglia, Serena Reggi**; writing original draft preparation: **Matteo Dell'Anno, Elena Scaglia**; writing review and editing: **Matteo Dell'Anno, Elena Scaglia, Silvia Grossi, Sara Frazzini, Serena Reggi, Luciana Rossi**; visualisation: **Matteo Dell'Anno**; supervision: **Luciana Rossi, Carlo Angelo Sgoifo Rossi**; funding acquisition: **Luciana Rossi**.

Declaration of interest

All authors declare no conflict of interests.

Acknowledgements

Thanks to the New Feed Team Srl (Lody, Italy) for providing the feed additive used in this study. The authors acknowledge the support of the APC central fund of the University of Milan.

Financial support statement

This study was supported by LEGUPLUS project funded by the Italian Ministry of Agricultural, Food and Forestry Policies (DM n. 9358195).

References

Amaral-Phillips, D.M., Scharko, P.B., Johns, J.T., Franklin, S., 2006. Feeding and managing baby calves from birth to 3 months of age. UK Cooperative Extension Service, University of Kentucky, College of Agriculture, Lexington, KY, USA.
AOAC, 2019. Official Methods of Analysis. Association of Official Analytical Chemists, Washington, DC, USA.
Araujo, G., Terré, M., Mereu, A., Ipharraguerre, I., Bach, A., 2015. Effects of supplementing a milk replacer with sodium butyrate or tributyrin on performance and metabolism of Holstein calves. Animal Production Science 56, 1834–1841.
Berni Canani, R., Di Costanzo, M., Leone, L., 2012. The epigenetic effects of butyrate: potential therapeutic implications for clinical practice. Clinical Epigenetics 4, 1–7.
Brunauer, M., Roch, F.-F., Conrady, B., 2021. Prevalence of worldwide neonatal calf diarrhoea caused by bovine rotavirus in combination with Bovine Coronavirus, Escherichia coli K99 and Cryptosporidium spp.: a meta-analysis. Animals 11, 1014.
Cai, J., Chen, H., Weng, M., Jiang, S., Gao, J., 2019. Diagnostic and clinical significance of serum levels of D-lactate and diamine oxidase in patients with Crohn's disease. Gastroenterology Research and Practice 2019, 1–7.

Cho, Y.-I., Yoon, K.-J., 2014. An overview of calf diarrhea-infectious etiology, diagnosis, and intervention. Journal of Veterinary Science 15, 1–17.
Crawford, D.M., Richeson, J.T., Perkins, T.L., Samuelson, K.L., 2022. Feeding a high energy finishing diet upon arrival to high-risk feedlot calves: effects on health, performance, ruminal pH, rumination, serum metabolites, and carcass traits. Journal of Animal Science 100, 1–12.
Cresci, G.A., Glueck, B., McMullen, M.R., Xin, W., Allende, D., Nagy, L.E., 2017. Prophylactic tributyrin treatment mitigates chronic-binge ethanol-induced intestinal barrier and liver injury. Journal of Gastroenterology and Hepatology 32, 1587–1597.
Dell'Anno, M., Reggi, S., Caprarulo, V., Hejna, M., Sgoifo Rossi, C.A., Callegari, M.L., Baldi, A., Rossi, L., 2021. Evaluation of Tannin Extracts, Leonardite and Tributyrin Supplementation on Diarrhoea Incidence and Gut Microbiota of Weaned Piglets. Animals 11, 1693.
Dong, L., Zhong, X., He, J., Zhang, L., Bai, K., Xu, W., Wang, T., Huang, X., 2016. Supplementation of tributyrin improves the growth and intestinal digestive and barrier functions in intrauterine growth-restricted piglets. Clinical Nutrition 35, 399–407.
Du, K., Bereswill, S., Heimesaat, M.M., 2021. A literature survey on antimicrobial and immune-modulatory effects of butyrate revealing non-antibiotic approaches to tackle bacterial infections. European Journal of Microbiology and Immunology 11, 1–9.
Eibl, C., Bexiga, R., Viora, L., Guyot, H., Félix, J., Wilms, J., Tichy, A., Hund, A., 2021. The Antibiotic Treatment of Calf Diarrhea in Four European Countries: A Survey. Antibiotics 10, 910.
European Parliament, 2003. Regulation (EC) No. 1831/2003 of the European Parliament and of the Council of 22 September 2003 on Additives for Use in Animal Nutrition. Official Journal of the European Union, Brussels, Belgium.
European Medicines Agency (EMA), 2020. Categorisation of antibiotics used in animals promotes responsible use to protect public and animal health. EMA/688114/2020.
Frieten, D., Gerbert, C., Koch, C., Dusel, G., Eder, K., Kanitz, E., Weitzel, J., Hammon, H., 2017. Ad libitum milk replacer feeding, but not butyrate supplementation, affects growth performance as well as metabolic and endocrine traits in Holstein calves. Journal of Dairy Science 100, 6648–6661.
Fukuda, T., Tsukano, K., Nakatsuji, H., Suzuki, K., 2019. Plasma diamine oxidase activity decline with diarrhea severity in calves indicating systemic dysfunction related to intestinal mucosal damage. Research in Veterinary Science 126, 127–130.
Fukudome, I., Kobayashi, M., Dabanaka, K., Maeda, H., Okamoto, K., Okabayashi, T., Baba, R., Kumagai, N., Oba, K., Fujita, M., 2014. Diamine oxidase as a marker of intestinal mucosal injury and the effect of soluble dietary fiber on gastrointestinal tract toxicity after intravenous 5-fluorouracil treatment in rats. Medical Molecular Morphology 47, 100–107.
Gomez, D., Arroyo, L., Costa, M., Viel, L., Weese, J., 2017. Characterization of the fecal bacterial microbiota of healthy and diarrheic dairy calves. Journal of Veterinary Internal Medicine 31, 928–939.
Gong, L., Xiao, G., Zheng, L., Yan, X., Qi, Q., Zhu, C., Feng, X., Huang, W., Zhang, H., 2021. Effects of Dietary Tributyrin on Growth Performance, Biochemical Indices, and Intestinal Microbiota of Yellow-Feathered Broilers. Animals 11, 3425.
Górka, P., Kowalski, Z., Zabielski, R., Guilloteau, P., 2018. Invited review: Use of butyrate to promote gastrointestinal tract development in calves. Journal of Dairy Science 101, 4785–4800.
Grossi, S., Dell'Anno, M., Rossi, L., Compiani, R., Sgoifo Rossi, C.A., 2021. Supplementation of Live Yeast, Mannan Oligosaccharide, and Organic Selenium during the Adaptation Phase of Newly Arrived Beef Cattle: Effects on Health Status, Immune Functionality, and Growth Performance. Antibiotics 10, 1114.
Guilloteau, P., Zabielski, R., David, J.-C., Blum, J.W., Morisset, J.A., Biernat, M., Woliński, J., Laubitz, D., Hamon, Y., 2009. Sodium-butyrate as a growth promoter in milk replacer formula for young calves. Journal of Dairy Science 92, 1038–1049.
Guilloteau, P., Martin, L., Eeckhaut, V., Ducatelle, R., Zabielski, R., Van Immerseel, F., 2010. From the gut to the peripheral tissues: the multiple effects of butyrate. Nutrition Research Reviews 23, 366–384.
Hansen, V.L., Kahl, S., Proszkowiec-Weglarz, M., Jiménez, S.C., Vaessen, S.F., Schreier, L.L., Jenkins, M.C., Russell, B., Miska, K.B., 2021. The effects of tributyrin supplementation on weight gain and intestinal gene expression in broiler chickens during Eimeria maxima-induced coccidiosis. Poultry Science 100, 100984.
Hatew, B., Inabu, Y., Sugino, T., Steele, M., 2019. Effects of pulse-dose ruminal infusion of butyrate on plasma glucagon-like peptide 1 and 2 concentrations in dairy calves. Journal of Dairy Science 102, 2254–2265.
Hill, T., Aldrich, J., Schlotterbeck, R., Bateman II, H., 2007. Effects of changing the fat and fatty acid composition of milk replacers fed to neonatal calves. The Professional Animal Scientist 23, 135–143.
Hou, Y., Wang, L., Yi, D., Ding, B., Chen, X., Wang, Q., Zhu, H., Liu, Y., Yin, Y., Gong, J., Wu, G., 2014. Dietary supplementation with tributyrin alleviates intestinal injury in piglets challenged with intrarectal administration of acetic acid. The British Journal of Nutrition 111, 1748–1758.
Inabu, Y., Murayama, K., Inouchi, K., Sugino, T., 2019. The effect of tributyrin supplementation to milk replacer on plasma glucagon-like peptide 2 concentrations in pre-weaning calves. Animal Science Journal 90, 1185–1192.
Karamzadeh-Dehaghani, A., Towhidi, A., Zhandi, M., Mojgani, N., Fouladi-Nashta, A., 2021. Combined effect of probiotics and specific immunoglobulin Y directed against Escherichia coli on growth performance, diarrhea incidence, and immune system in calves. Animal 15, 100124.
Khan, D., Vasu, S., Moffett, R.C., Irwin, N., Flatt, P.R., 2017. Differential expression of glucagon-like peptide-2 (GLP-2) is involved in pancreatic islet cell adaptations to stress and beta-cell survival. Peptides 95, 68–75.
Khawaskar, D.P., Sinha, D., Lalrinzuala, M.V., Athira, V., Kumar, M., Chhakchhuak, L., Mohanapriya, K., Sophia, I., Kumar, O., Chaudhuri, P., 2021. Pathotyping and antimicrobial susceptibility testing of Escherichia coli isolates from neonatal calves. Veterinary Research Communications 46, 353–362.
Kovanda, L., Zhang, W., Wei, X., Luo, J., Wu, X., Atwill, E.R., Vaessen, S., Li, X., Liu, Y., 2019. In vitro antimicrobial activities of organic acids and their derivatives on several species of gram-negative and gram-positive bacteria. Molecules 24, 3770.
Leonel, A.J., Teixeira, L.G., Oliveira, R.P., Santiago, A.F., Batista, N.V., Ferreira, T.R., Santos, R.C., Cardoso, V.N., Cara, D.C., Faria, A.M., 2013. Antioxidative and immunomodulatory effects of tributyrin supplementation on experimental colitis. The British Journal of Nutrition 109, 1396–1407.
Li, F.-C., Li, Y.-K., Fan, Y.-C., Wang, K., 2018. Plasma concentration of diamine oxidase (DAO) predicts 1-month mortality of acute-on-chronic hepatitis B liver failure. Clinica Chimica Acta 484, 164–170.
Liu, Y., Chen, F., Odle, J., Lin, X., Jacobi, S.K., Zhu, H., Wu, Z., Hou, Y., 2012. Fish oil enhances intestinal integrity and inhibits TLR4 and NOD2 signaling pathways in weaned pigs after LPS challenge. The Journal of Nutrition 142, 2017–2024.
Liu, W., La, A., Evans, A., Gao, S., Yu, Z., Bu, D., Ma, L., 2021b. Supplementation with sodium butyrate improves growth and antioxidant function in dairy calves before weaning. Journal of Animal Science and Biotechnology 12, 1–9.
Liu, S., Ma, J., Zhou, J., Wu, J., Li, J., Alugongo, G., Xiao, J., Wang, J., Wang, Y., Wang, W., 2021a. Tributyrin supplementation in pasteurized waste milk: Effects on growth performance, health, and blood parameters of dairy calves. Journal of Dairy Science 104, 12496–12507.
Liu, S., Wu, J., Wu, Z., Alugongo, G.M., Khan, M.Z., Li, J., Xiao, J., He, Z., Ma, Y., Li, S., 2022. Tributyrin administration improves intestinal development and health in pre-weaned dairy calves fed milk replacer. Animal Nutrition 10, 399–411.
Miragoli, F., Patrone, V., Prandini, A., Sigolo, S., Dell'Anno, M., Rossi, L., Senizza, A., Morelli, L., Callegari, M.L., 2021. Implications of Tributyrin on Gut Microbiota Shifts Related to Performances of Weaning Piglets. Microorganisms 9, 584.
Mutanen, A., Pakarinen, M.P., 2017. Serum fasting GLP-1 and GLP-2 associate with intestinal adaptation in pediatric onset intestinal failure. Clinical Nutrition 36, 1349–1354.
Namkung, H., Yu, H., Gong, J., Leeson, S., 2011. Antimicrobial activity of butyrate glycerides toward Salmonella Typhimurium and Clostridium perfringens. Poultry Science 90, 2217–2222.
Neath, C., Portocarero, N., Jones, C., 2022. In vitro susceptibility of swine pathogens to feed additives and active ingredients with potential as antibiotic replacements. Journal of Applied Microbiology 132, 1713–1723.
O'Hara, E., Kelly, A., McCabe, M.S., Kenny, D.A., Guan, L.L., Waters, S.M., 2018. Effect of a butyrate-fortified milk replacer on gastrointestinal microbiota and products of fermentation in artificially reared dairy calves at weaning. Scientific Reports 8, 1–11.
Pyo, J., Hare, K., Pletts, S., Inabu, Y., Haines, D., Sugino, T., Guan, L., Steele, M., 2020. Feeding colostrum or a 1: 1 colostrum: milk mixture for 3 days postnatal increases small intestinal development and minimally influences plasma glucagon-like peptide-2 and serum insulin-like growth factor-1 concentrations in Holstein bull calves. Journal of Dairy Science 103, 4236–4251.
Ren, S., Chen, A., Tian, Y., Bai, Z., Wang, C., 2022. Lactobacillus paracasei from Koumiss Ameliorates Diarrhea in mice via Tight Junctions Modulation. Nutrition 98, 111584.
Rossi, L., Turin, L., Alborali, G.L., Demartini, E., Filipe, J.F.S., Riva, F., Riccaboni, P., Scanziani, E., Trevisi, P., Dall'Ara, P., 2021. Translational Approach to Induce and Evaluate Verocytotoxic E. coli O138 Based Disease in Piglets. Animals 11, 2415.
Santos, F., De Paula, M., Lezier, D., Silva, J., Santos, G., Bittar, C., 2015. Essential oils for dairy calves: effects on performance, scours, rumen fermentation and intestinal fauna. Animal 9, 958–965.
Sotira, S., Dell'Anno, M., Caprarulo, V., Hejna, M., Pirrone, F., Callegari, M.L., Tucci, T. V., Rossi, L., 2020. Effects of Tributyrin Supplementation on Growth Performance, Insulin, Blood Metabolites and Gut Microbiota in Weaned Piglets. Animals 10, 726.
Terré, M., Ortuzar, I., Graffelman, J., Bassols, A., Vidal, M., Bach, A., 2021. Using compositional mixed-effects models to evaluate responses to amino acid supplementation in milk replacers for calves. Journal of Dairy Science 104, 7808–7819.
Waguespack, A., Powell, S., Roux, M., Fruge, E., Bidner, T., Payne, R., Southern, L., 2011. Effect of determining baseline plasma urea nitrogen concentrations on subsequent posttreatment plasma urea nitrogen concentrations in 20-to 50-kilogram pigs. Journal of Animal Science 89, 4116–4119.
Windeyer, M., Leslie, K., Godden, S.M., Hodgins, D., Lissemore, K., LeBlanc, S., 2014. Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. Preventive Veterinary Medicine 113, 231–240.
World Health Organization (WHO), 2019. Critically important antimicrobials for human medicine. 6th Revision 2018. Ranking of medically important antimicrobials for risk management of antimicrobial resistance due to non-human use. Switzerland.
Xie, D., Dai, Q., Xu, C., Li, Y., 2021. Dietary tributyrin modifies intestinal function by altering morphology, gene expression and microbiota profile in common carp (Cyprinus carpio) fed all-plant diets. Aquaculture Nutrition 27, 439–453.

Zhang, Z., Zhang, G., Zhang, S., Zhao, J., 2022. Fructooligosaccharide Reduces Weanling Pig Diarrhea in Conjunction with Improving Intestinal Antioxidase Activity and Tight Junction Protein Expression. Nutrients 14, 512.

Zhou, Z., Packialakshmi, B., Makkar, S., Dridi, S., Rath, N., 2014. Effect of butyrate on immune response of a chicken macrophage cell line. Veterinary Immunology and Immunopathology 162, 24–32.