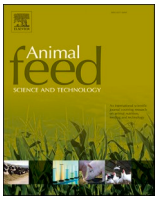
Animal Feed Science and Technology 321 (2025) 116243

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci



Effects of feed additives in the diet of male dairy beef calves on physiological status and rumen microbial fermentation pre- and postweaning

E. Romera-Recio [a], E. Ramos-Morales [a], A. Belanche [b], M. Hassan [a], P. Romero [a,c], A. Gómez [a], I. Rivelli [a], N. Llanes [d], J. Torra [d], D.R. Yáñez-Ruiz [a,*]

[a] Estación Experimental del Zaidín (CSIC), Profesor Albareda, 1, Granada 18008, Spain
[b] Departamento de Producción Animal y Ciencia de los Alimentos, Universidad de Zaragoza, Miguel Servet 177, Zaragoza 50013, Spain
[c] Department of Animal Science, University of California, Davis, CA 95616, USA
[d] Ivars d'Urgell Cooperativa del Camp, Lleida, Spain

ARTICLE INFO

Keywords:
Calves
Feed additives
Rumen development
Weaning

ABSTRACT

In Spain, a large number of unweaned calves from northern Europe's dairy industry are transported for intensive beef production, which could pose health risks around weaning due to the separation from their mothers. This study evaluated the impact of including different feed additives in starter concentrate on blood parameters and rumen functional development in 112 calves at the growing farm. We hypothesized that feed additives could enhance rumen function and mitigate health risks associated with transportation and intensive management. The treatments tested, against a control diet (**CTL**), included: blend of essential oils from plant extracts (**EO**), yeast-based products (**SYN**) and a mix of yeast probiotics, oregano-based essential oil and sodium butyrate (**MIX**). Each treatment was administered during the preweaning stage, with all calves transitioning to the MIX diet postweaning. In the experiment, blood and rumen samples were collected before weaning (8 weeks old) and two months after weaning (18 weeks old) for rumen fermentation and microbial population analyses. Calves were weighed upon arrival (3 weeks), at weaning (10 weeks) and two months postweaning (18 weeks) to assess performance. Results from the experiment showed that rumen fermentation profiles remained stable with regard to volatile fatty acids (**VFA**) concentrations and pH, indicating effective solid feed consumption and microbial activity before weaning. Postweaning, all feed additives treatments improved rumen fermentation by increasing total VFA and lowering pH, though body weight gains remained unaffected. Concentrations of bacteria and archaea increased compared to preweaning levels, protozoa were absent, and anaerobic fungi did not become established until 2 months postweaning. In conclusion, feed additives provided preweaning improved rumen

Abbreviations: A:, P, Acetate:propionate ratio; ADG, Average daily gain; BHB, Beta-hydroxybutyrate; BUN, Blood urea nitrogen; BCVFA, Branched-chain volatile fatty acids; CK, Creatin kinase; CREA, Creatinine; DM, Dry matter; EO, Essential Oil; GC, Gas Chromatography; GGT, Gamma-glutamyl transferase; GOT, Glutamic-oxaloacetic transaminase; GPT, Glutamic-pyruvate transaminase; HCT, Haematocrit; HDL, High-density lipoproteins; HGB, Haemoglobin; Hp, Haptoglobin; IgG, Immunoglobulin G; LDH, Lactate dehydrogenase; LDL, Low-density lipoproteins; MIX, Mixed diet; NEFA, Non-esterified fatty acids; SEM, Standard error of the mean; SYN, Synbiotic; TADG, Total average daily gain; TG, Triglycerides; VFA, Volatile fatty acids; WBC, White blood cells.

* Corresponding author.
E-mail address: david.yanez@eez.csic.es (D.R. Yáñez-Ruiz).

https://doi.org/10.1016/j.anifeedsci.2025.116243
Received 6 October 2024; Received in revised form 20 December 2024; Accepted 25 January 2025
Available online 31 January 2025

development, although they did not increase productivity. The treatment should be applied preweaning, highlighting the importance of this particular window of time.

1. Introduction

In 2022, the European Union produced 6.6 million tonnes of bovine meat, with France, Germany, Italy and Spain contributing, respectively with 20.8 %, 16.5 %, 11.6 % and 11.0 % (Eurostat, 2022). Annually, Spain receives around 700,000 unweaned calves from EU dairy farms for growth and fattening, with France supplying the majority (64.2 %).

After transportation to the final rearing farm and acclimatization to the environment, one of the challenges calves face is to increasingly achieving solid feed intake in preparation for weaning (Devant and Marti, 2020). This can be hindered by the difficulty of acquiring an optimal rumen microbial, functional and anatomical development in a system where calves had been separated from the dams immediately after birth. At birth and during the first weeks of life, the rumen is not fully developed and lacks the microbiota present in adult animals (Abecia et al., 2014a). A correct transition from proto-rumen to rumen will later determine the efficiency of the nutrients' digestion and absorption in the gut and other tissues (Baldwin et al., 2004). In this transition, microbial colonization occurring after birth plays a pivotal role on the development of the rumen that undergoes dramatic changes through the first weeks of life up to weaning and beyond (Rey et al., 2014). Previous works have reported the unique and potentially important influences of maternal microbiota from the faeces, udder, vagina, saliva and colostrum, which each appears to make early contributions to the bio-spatial and longitudinal succession of microbes throughout the early life of the animal (Yeoman et al., 2018). This is particularly critical in the context of dairy livestock systems in which the newborn is taken away from the mother after birth, generally fed on artificial milk, and are kept isolated from adult animals, which can limit the rumen microbial development and animal performance (Belanche et al., 2019).

Numerous studies and approaches attempt to modulate rumen fermentation and the microbial community in young ruminants to optimize rumen development. These approaches include alteration of diet composition and physical forms, introduction of variables in the feeding management and addition of new types of feed additives (Diao et al., 2019). The use of feed additives not only can promote an adequate rumen function development but also minimize the health risks associated to transportation and non-optimal management at the assembly centers. Numerous types of feed additives have been tested in calves, including plant secondary compounds and probiotics (Salazar et al., 2019; Stefańska et al., 2021). However, until now, the effects of treatments with plant extracts or/and probiotics have not been consistent on the performance of preweaned dairy calves. This is most likely due to variations in dosage, the strain composition of the probiotics and the chemical profile and concentrations of the bioactive compounds in the extracts, as well as the use of different ration compositions and animal management strategies across systems and regions.

The aim of this study was to evaluate the impact of including feed additives with different modes of action in the concentrate feed of male dairy calves on blood parameters, rumen microbial fermentation and colonization at weaning and postweaning stages.

2. Material and methods

The experiment studied the effect of providing different feed additives on body weight gain, blood parameters, rumen fermentation and microbial numbers in calves at weaning and then the residual effects two months after weaning.

2.1. Experimental animals

All the experimental procedures involved in this study were performed by trained personnel in accordance with Spanish guidelines (RD 53/2013) and approved by the Ethical Committee for Animal Research (EEZ-CSIC) of the regional government (ref. 1022/2020).

A total of 112 Montbéliarde male suckling calves, aged 21 ± 7 days (mean ± SD) from diverse French dairy farms were randomly selected at the assembly farm centre of "Les veaux des frères Drevon", in Saint-Sulpice-des-Rivoires, France. The calves were assembled at the reception facility before embarking on a 750 km and 12-hour journey from the Saint-Sulpice-des-Rivoires region in France (coordinates 45.461 N, 5.591 E, and 511 m altitude) to the Cooperative d'Ivars in Ivars d'Urgell (Spain, coordinates 41.715 N, 0.995 E, and 275 m altitude). The transportation took place in October 2022. Calves were fed milk replacer 4 hours before departure at the origin and had access to water during the rest hour (after 9 hours of travel). The vehicle provided a space allowance of 0.4 m^2 per animal and were equipped with straw bedding to enhance comfort during the journey.

Upon arrival at the farm, the calves were allowed time to stabilize and calm down before being sampled. After blood sampling, each calf received a 2 L rehydration solution (Hydrabas) with a total solute concentration of 50 g/L, consisting of 700 mg glucose monohydrate, 100 mg sodium chloride, 40 mg potassium chloride, 100 mg sodium bicarbonate, 50 mg sodium citrate, and 10 mg orange extract. Each animal was weighed at three specific times: upon arrival (3 weeks of age), at weaning (10 weeks of age), and two months postweaning (18 weeks of age).

Calves were fed twice daily (0730 and 1700 h) with 4 L of milk replacer (120 g powder/L milk replacer, Sprayfo Excellent) from arrival to 7 weeks of age, then transitioned to once-daily feeding of 2 L milk replacer in the morning until weaning at 10 weeks of age. At 10 weeks of age, milk replacer was no longer provided and therefore the postweaning phase started. The powdered milk replacer's composition included: 30 % skimmed milk powder, 22.5 % crude protein, 18 % crude oils and fats, 8 % crude ash, and 0 % crude fibre. Additionally, the replacer provided 25,000 IU of vitamin A, 5000 IU of vitamin D3, 300 mg of vitamin E, 0.3 mg of selenium, 10 mg of

copper, and 90 mg of iron. In addition to milk replacer, animals had ad libitum access to oat hay and concentrate, offered from the first day of arrival at the farm and refreshed daily. Any refused concentrate was removed. However, the individual feed intake of the animals could not be measured. The concentrate consisted of a pre-starter ground feed (1.1 kg per animal/day for 30 days, from week 3 to week 8 of age) followed by a pellet-format starter (2 kg per animal/day for 60 days, covering weeks 9–18 of age). Samples of the concentrate feeds and milk replacer were collected weekly, stored at −20ºC and then pooled for compositional analyses.

The nutritional composition of the pre-starter ground feed (g/100 g DM) was as follows: 17.8 crude protein, 3.64 crude fat, 4.40 crude fibre, 4.65 crude ash, 0.57 calcium, 0.43 phosphorus, 0.24 sodium, and 0.64 magnesium. Additionally, it provided 10,000 IU of vitamin A, 2000 IU of vitamin D3, and 50 mg/kg of vitamin E. The nutritional composition of the starter pellet composition (g/100 g DM) included: 14.8 crude protein, 3.40 crude fat, 3.53 crude fibre, 5.52 crude ash, 0.96 calcium, 0.43 total phosphorus, 0.24 sodium, and 0.64 magnesium. It also contained 10,000 IU of vitamin A, 2000 IU of vitamin D3, and 75 mg/kg of vitamin E. The trace element composition (mg/kg) for both diets was as follows: 52 iron (Fe), 10 copper (Cu), 35 zinc (Zn), 0.3 selenium (Se), 0.4 cobalt (Co), 0.6 iodine (I), and 30 manganese (Mn). Both concentrate feeds (ground and pelleted) also included 0.6 mg/kg of butylated hydroxytoluene (BHT).

Animals were randomly allocated to one of the following experimental treatments (n = 28 per group): a control diet (**CTL**) without any additives and three experimental diets with treatments included in the concentrate: Essential Oil (**EO**) diet containing a blend of essential oils and plant extracts (primarily of, oregano oil, citrus oil, anise oil, maltol, sodium chloride, and silicon dioxide, DSM-firmenich, Switzerland) at a dosage of 300 g/ton in both pre-starter and starter feeds; a Synbiotic (**SYN**) diet containing a combination of inactivated yeast strains from YANG and Levucell (Lallemand Inc. Animal Nutrition, Canada) at a dosage of 1.5 kg/ton for YANG and 100 g/ton for Levucell in pre-starter feed, and 1 kg/ton for YANG and 100 g/ton for Levucell in starter feed; and a Mixed (**MIX**) diet consisting of a combination of the following products: yeast probiotics, oregano-based essential oil, and sodium butyrate. The feed additives were included into the feed during the manufacturing process at the d'Ivars company's feed factory.

Animals received their corresponding treatments upon arrival to the rearing farm after the transportation and for 45 days until weaning (from 3 to 10 weeks of age). After weaning, all animals received the MIX diet. Blood and rumen samples from 112 calves were collected after 1.5 hours of fasting in the morning, before weaning (day 35 post-arrival, week 8 of age) and at 2 months postweaning (day 105 post-arrival, week 18 of age). Blood samples were obtained by jugular venepuncture into 10 mL EDTA K2, and silicone dry Vacutainer tubes, and analysed for biochemical and haematological parameters. Rumen fluid (approximately 50 mL) was obtained using an oral probe attached to a vacuum pump and strained through two layers of cheesecloth, as described by Ramos-Morales et al. (2014). The pH was immediately measured and subsamples were collected for VFA determination as follows: 0.8 mL were diluted with 0.8 mL of an acid solution (0.5 N HCl, 20 g/L metaphosphoric acid containing 0.8 g/L of crotonic acid as internal standard) and stored at −20ºC. For lactate analysis, 1.6 mL rumen fluid were diluted with 0.4 mL of trichloroacetate solution (250 g/L) and stored at −20ºC. For microbial quantification, 30 mL were immediately frozen in liquid N prior storage at −80ºC.

2.2. Samples analysis

2.2.1. Feed compositional analysis

Samples of the pre-starter ground feed and starter pellet feed were collected weekly throughout the study period, pooled to form representative samples, and stored at −20°C until analysis. The nutritional composition of these samples was determined following standard analytical methods as described in Arco-Pérez et al. (2017). Dry matter (DM) content was measured according to AOAC method 934.01, and ash content was determined by incineration at 550°C using method 942.05 (AOAC, 2005). Crude protein (CP) was quantified using the Kjeldahl method, while crude fat was assessed by Soxhlet extraction. The crude fiber content was determined using the Van Soest detergent fiber method, with neutral detergent fiber (NDF) and acid detergent fiber (ADF) calculated using the methods of Van Soest et al. (1991). Lignin content (ADL) was determined by solubilization with 72 % sulfuric acid. Mineral concentrations, including calcium, phosphorus, sodium, and magnesium, were measured using atomic absorption spectrophotometry. Vitamin A, D3, and E were quantified by high-performance liquid chromatography (HPLC). Trace elements, such as iron (Fe), copper (Cu), zinc (Zn), selenium (Se), cobalt (Co), iodine (I), and manganese (Mn), were analyzed by inductively coupled plasma mass spectrometry (ICP-MS). Additionally, the presence of butylated hydroxytoluene (BHT) at 0.6 mg/kg was confirmed using gas chromatography with a flame ionization detector (GC-FID) as described by Arco-Pérez et al. (2017).

2.2.2. Blood parameters

The analysis of blood parameters was carried out at the Gasset Veterinary Laboratory (Granada, Spain). After collection, the tubes were kept refrigerated and then centrifuged at 3000 rpm for 15 minutes at 4ºC to obtain the serum. Haematological parameters were determined using an XN-V 1500 fluorescence flow cytometry analyser provided by Sysmex Corporation (Kobe, Japan). Whole blood was analysed for white blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), and platelet counts using a VetScan HM2 (Diamond Diagnostics, Abaxis Inc., Holliston, MA, USA).

To determine biochemical metabolites in serum, including glucose and insulin, as well as glutamic-oxaloacetic transaminase (GOT), gamma-glutamyl transferase (GGT), and glutamic-pyruvate transaminase (GPT) were quantified using specific kits (LABTEST, Brazil) by spectrophotometry in a semi-automatic biochemical analyser (Cobas C111, Roche). GOT and GPT activities were assessed by monitoring the oxidation of NADH at 340 nm, while GGT activity was determined by measuring the release of p-nitroaniline at 405 nm (Brunel et al., 2018).

Wako Pure Chemical (Osaka, Japan) provided the analytical reagents to measure the levels of non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), blood urea nitrogen (BUN), triglycerides (TG), creatinine (CREA), total bilirubin, cholesterol, high-

density lipoproteins (HDL), and low-density lipoproteins (LDL), total protein, sodium (Na), potassium (K), chloride (Cl^-), amylase, lactate dehydrogenase (LDH), and creatin kinase (CK), various techniques including photometry, absorbance, and turbidimetry were employed using a SPIN 640 plus automated chemical analyser provided by SPINREACT, S.A. (Toyobo, Japan).

Additionally, commercial bovine ELISA test kits (Life Diagnostics, Inc., West Chester, PA, USA; Endocrine Technologies, Inc., Newark, CA, USA) were employed to assess the levels of serum cortisol, haptoglobin (Hp), albumin and globulins.

2.2.3. Rumen fermentation

Volatile fatty acids were determined using a Gas Chromatography (GC) system Focus (Thermo, Rodano, Milan-Italy) coupled with a Triplus AS autosampler (Thermo, Rodano, Milan-Italy) with a crosslinked 100 % polyethylene glycol column (TRB-FFAP, 30 m 0.53 mm i.d., 1 μm film thickness, Teknokroma, Spain), as described by Arco-Pérez et al. (2017). Lactate concentration was determined by colorimetry based on the procedures described by Barker and Summerson (1941).

2.2.4. DNA extraction and quantitative PCR

Total genomic DNA extraction from frozen samples was performed using the DNeasy PowerSoil Pro Kit (Qiagen, Germany) following the manufacturer's instructions. Absolute concentrations of DNA from total bacteria, methanogens, fungi and protozoa were determined by qPCR using serial dilutions of their respective standards (10^{-1}–10^{-5}) as previously described by Palma-Hidalgo et al. (2021).

2.3. Calculations and statistical analyses

Prior conducting the analyses, the assumptions of normality and homogeneity of variance were checked using the Shapiro-Wilk and Bartlett's tests, respectively. The results were analysed using RStudio™ software (R version 4.3.1, Boston, MA).

In the experiment, a generalized linear model (GLM) was utilized to discern significant differences for each variable (weights, blood parameters, rumen fermentation parameters and microbial populations) across treatments, the age of animals being considered as a covariate. Statistical significance was set at $P < 0.05$, while trends were considered within $0.05 \leq P < 0.10$. The following model was used:

Table 1
Effect of feed additive nutritional intervention on blood biochemistry parameters in male calves before weaning.

Parameter	Treatment				SEM	P-value
	CTL[1]	EO[2]	SYN[3]	MIX[4]		
IgG (g/dL)	3.16	2.04	1.77	2.76	0.49	0.185
Haptoglobin (mg/dL)	1.37	0.56	0.73	0.55	0.31	0.216
Cortisol (μg/dL)	1.30	1.18	1.23	1.34	0.90	0.607
Glucose (mg/dL)	58.8	51.3	52.3	60.6	2.93	0.064
Insulin (μU/mL)	2.03	2.00	2.00	2.00	0.01	0.519
Cholesterol (mg/dL)	54.9	55.1	56.2	60.0	2.68	0.510
HDL Cholesterol (mg/dL)	36.3	38.5	38.3	38.8	1.47	0.641
LDL Cholesterol (mg/dL)	18.5	16.4	18.0	21.2	1.66	0.226
NEFA (mmol/L)	0.22	0.17	0.18	0.21	0.02	0.086
BHB (mg/dL)	2.33^{c}	3.20^{a}	2.51^{bc}	2.88^{ab}	0.18	0.004
LDH (U/L)	361	379	435	278	41.6	0.067
CK (U/L)	153^{ab}	111^{b}	133^{b}	204^{a}	21.6	0.022
GOT (U/L)	66.1^{ab}	59.4^{b}	59.2^{b}	69.0^{a}	2.50	0.012
GPT (U/L)	17.6	15.8	14.3	18.6	1.49	0.183
GGT (U/L)	28.9	27.4	28.0	26.6	2.09	0.888
Amylase (U/L)	45.2	38.7	42.2	42.2	2.27	0.264
Urea (mg/dL)	13.7	12.1	11.3	13.5	1.22	0.456
Creatinine (mg/dL)	0.87	0.94	0.93	0.99	0.03	0.088
Total Bilirubin (mg/dL)	0.29	0.24	0.24	0.34	0.05	0.482
Total Protein (g/dL)	6.19	5.96	5.92	6.14	0.09	0.091
Albumin (g/dL)	3.45	3.45	3.48	3.51	0.03	0.600
Globulins (g/dL)	2.73	2.53	2.44	2.62	0.09	0.111
Sodium (mEq/L)	145^{b}	146^{b}	148^{a}	147^{ab}	0.74	0.034
Potassium (mEq/L)	6.58	6.12	5.90	6.44	0.20	0.084
Chloride (mEq/L)	98.0	96.2	97.4	98.7	0.86	0.197

Abbreviations: IgG = Immunoglobulin G; LDH = Lactate Dehydrogenase; CK = Creatine kinase; GOT = Glutamic-oxaloacetic transaminase; GGT = Gamma-glutamyl transferase; GPT: Glutamic-pyruvate transaminase; HDL = High-Density Lipoprotein Cholesterol; LDL = Low-Density Lipoprotein Cholesterol; NEFA = Non-Esterified Fatty Acid; BHB = Beta-Hidroxybutyrate.

[1] CTL: Control Diet with no additives.

[2] EO: Essential Oil Diet containing Digestarom from DSM Nutritional Products, Switzerland.

[3] SYN: Synbiotic Diet containing YANG and Levucell from Lallemand Inc. Animal Nutrition, Canada.

[4] MIX Mixed Diet a combination of probiotics, yeast fractions, oregano-based essential oil, and sodium butyrate.

a,b,c Values within a row with different superscripts differ significantly at $P < 0.05$.

$$Yij = \mu + \tau i + \beta(Xij - \overline{X}) + \epsilon ij$$

Where *Yij* represented the observation of the dependent variable for treatment *i* and observation *j;* μ was the intercept, denoting the overall mean of each dependent variable; τi meant the effect of treatment *i* on the dependent variable; β was the coefficient representing the association between age and the dependent variable, with *Xij* being the age value for treatment *i* in observation *j*; and $\overline{X}$ the mean age in the dataset; and finally, ϵij represented the random error associated with each treatment *i* and observation *j*.

3. Results

3.1. Blood parameters

Overall, small differences were observed in blood parameters across treatments in samples collected before weaning (Table 1). The MIX treatment showed significantly greater values for CK (204 U/L, $P = 0.022$), compared to both EO (111 U/L) and SYN (133 U/L) treatments. Similarly, GOT levels in animals fed the MIX treatment (69 U/L) were significantly higher ($P = 0.012$) than those of EO (59.4 U/L) and SYN (59.2 U/L) treatments.

There was a trend ($P = 0.064$) for glucose concentration to increase with CTL (58.8 mg/dL) and MIX (60.6 mg/dL) treatments, compared to EO and SYN (51.1 and 52.3 mg/dL, respectively). A similar trend was observed for NEFA ($P = 0.086$) with CTL and MIX having greater values (0.22 and 0.21 mmol/L, respectively) than EO and SYN (0.17 and 0.18 mmol/L, respectively). The concentration of BHB was significantly greater (P = 0.004) in animals receiving EO and MIX (3.20 and 2.88 mg/dL, respectively) compared to the CTL and SYN treatments (2.33 and 2.51 mg/dL).

Likewise, minor changes in blood electrolytes were observed between treatments, with the SYN diet showing slightly higher (P = 0.034) sodium levels compared to the CTL and EO treatments.

Compared to the sampling conducted at weaning, greater differences were observed in blood parameters across treatments in samples collected during the postweaning period (Table 2). Significant ($P = 0.032$) differences were observed in haptoglobin, with SYN showing lower levels (0.58 mg/dL) compared to the EO (1.36 mg/dL) and MIX (1.24 mg/dL) treatments. Serum cortisol levels were higher ($P < 0.001$) in the CTL group (2.37 μg/dL) compared to the groups that had received additives (from 1.15 to 1.78 μg/dL).

Table 2
Effect of feed additive nutritional intervention on blood biochemistry parameters in male calves at 2 months postweaning.

Parameter	Treatment				SEM	P-value
	CTL[1]	EO[2]	SYN[3]	MIX[4]		
IgG (g/dL)	4.15	4.51	3.91	4.21	0.43	0.794
Haptoglobin (mg/dL)	0.94[ab]	1.36[a]	0.58[b]	1.24[a]	0.20	0.032
Cortisol (μg/dL)	2.37[a]	1.61[bc]	1.15[c]	1.78[b]	0.18	< 0.001
Glucose (mg/dL)	28.2[ab]	32.2[a]	30.5[a]	20.3[b]	3.08	0.044
Insulin (μU/mL)	2.09[b]	2.07[b]	1.97[b]	4.80[a]	0.66	0.010
Cholesterol (mg/dL)	79.4	86.1	80.4	88.5	3.34	0.164
HDL Cholesterol (mg/dL)	41.5	43.7	43.4	41.3	1.62	0.698
LDL Cholesterol (mg/dL)	37.0[9b]	42.4[ab]	36.8[b]	47.2[a]	2.34	0.010
NEFA (mmol/L)	0.82	0.60	0.66	0.63	0.14	0.654
BHB (mg/dL)	3.07[c]	3.93[b]	4.68[a]	4.56[ab]	0.23	< 0.001
LDH (U/L)	2895	2905	2797	3066	72.9	0.089
CK (U/L)	432	386	255	473	59.8	0.069
GOT (U/L)	120.8	113.2	108.7	128.2	5.17	0.055
GPT (U/L)	21.8[b]	23.2[b]	23.2[b]	25.8[a]	0.91	0.026
GGT (U/L)	5.47	6.09	7.21	6.52	0.70	0.345
Amylase (U/L)	44.8	43.1	49.2	44.6	2.52	0.350
Urea (mg/dL)	18.8	18.6	17.3	18.9	0.94	0.576
Creatinine (mg/dL)	0.72[a]	0.66[a]	0.72[a]	0.52[b]	0.04	0.001
Total Bilirubin (mg/dL)	0.23	0.19	0.18	0.26	0.02	0.133
Total Protein (g/dL)	5.85[b]	5.85[b]	5.71[b]	6.35[a]	0.09	< 0.001
Albumin (g/dL)	3.50	3.53	3.52	3.55	0.04	0.889
Globulins (g/dL)	2.35[b]	2.32[b]	2.19[b]	2.80[a]	0.09	< 0.001
Sodium (mEq/L)	137.2[b]	137.5[b]	141.5[a]	136.5[b]	0.88	< 0.001
Potassium (mEq/L)	7.88	7.87	7.46	8.13	0.21	0.182
Chloride (mEq/L)	94.4	95.1	96.5	95.8	0.89	0.381

Abbreviations: IgG = Immunoglobulin G; LDH = Lactate Dehydrogenase; CK = Creatine kinase; GOT = Glutamic-oxaloacetic transaminase; GGT = Gamma-glutamyl transferase; GPT: Glutamic-pyruvate transaminase; HDL = High-Density Lipoprotein Cholesterol; LDL = Low-Density Lipoprotein Cholesterol; NEFA = Non-Esterified Fatty Acid; BHB = Beta-Hidroxybutyrate.

[1] CTL: Control Diet with no additives.

[2] EO: Essential Oil Diet containing Digestarom from DSM Nutritional Products, Switzerland.

[3] SYN: Synbiotic Diet containing YANG and Levucell from Lallemand Inc. Animal Nutrition, Canada.

[4] MIX Mixed Diet a combination of probiotics, yeast fractions, oregano-based essential oil, and sodium butyrate.

a,b,c Values within a row with different superscripts differ significantly at $P < 0.05$.

Glucose and insulin values were the lowest ($P = 0.044$) and highest ($P = 0.010$) for the MIX diet, compared to the rest of the treatments. Creatinine levels were significantly lower ($P = 0.001$) in the MIX group compared to the other groups. Concerning electrolytes, SYN treatment showed greater ($P < 0.05$) sodium values than any of the other treatments (141.5 mEq/L).

In terms of rumen activity indirect indicators, BHB was greater ($P < 0.001$) in animals that received the three different additives treatments compared to CTL, increasing by 28 %, 49 %, and 52 % with EO, MIX and SYN, respectively.

Additional information on haematological blood analysis before weaning and 2 months postweaning can be found in supplementary tables 1 and 2 respectively.

3.2. Rumen fermentation and quantitative PCR

Overall, fermentation parameters values were more homogeneous across treatments during the weaning period, compared to the postweaning period.

At weaning (Table 3), no significant differences were observed in rumen pH ($P = 0.169$) and VFA ($P = 0.222$) among treatments. As for the molar proportions of VFA, the MIX treatment caused a shift towards lower acetate ($P = 0.004$) and higher propionate ($P = 0.05$), resulting in a 13 % decrease in the ratio acetate to propionate ($P = 0.010$), compared to the control. The MIX treatment also caused an 18 % decrease in the molar proportion of branched-chain VFA.

Quantitative PCR showed no presence of fungi and protozoa and no differences for bacteria and archaeal abundances.

At the postweaning period (Table 4), both the SYN and the MIX treatments resulted in a slightly lower rumen pH (6.42 vs 6.83), compared to the control ($P = 0.019$). The concentration of total VFA was significantly higher ($P = 0.002$) for the MIX, SYN and EO treatments, with increases of 23, 24 and 34 %, respectively, in comparison with the control.

Regarding the molar proportions of volatile fatty acids, although acetate and propionate remained unchanged ($P > 0.05$), treatments MIX and EO increased ($P = 0.048$) the molar proportion of butyrate by 21 % and 24 %, respectively, in comparison with the CTL. Both SYN and MIX treatments promoted a decrease ($P = 0.001$) in molar proportion of BCVFA compared to the control, with the SYN treatment having a greater effect (38 %).

Quantitative PCR showed greater abundances of bacteria and archaea, and the presence of fungi, in comparison to those observed in the samples collected at weaning and still could not detect protozoal DNA. There was no significant effect of any of the treatments on the abundances of bacteria, archaea, or fungi.

3.3. Body weight gain

No differences between treatments were observed for any of the weights recorded ($P > 0.05$; Table 5). Likewise, the average daily gain (ADG), did not differ between experimental groups from arrival at the farm to weaning (ADG1) ($P = 0.190$) and in the period from weaning to postweaning (ADG2) ($P = 0.989$). As a result, the total ADG, covering the entire experimental period did not differ between treatments ($P = 0.889$).

Although there were no significant differences between treatments, a greater variability in weights within the CTL group compared to the treated animals was observed (SD for total ADG 216, 171, 121 and 178 g/day for CTL, MIX, EO and SYN respectively).

Table 3
Effect of feed additive nutritional intervention on rumen fermentation profile and microbial biomass (Log_{10} Copy/mL) before weaning.

Parameter	Treatment				SEM	P-value
	CTL[1]	EO[2]	SYN[3]	MIX[4]		
pH	6.13	6.19	6.49	5.94	0.17	0.169
Lactic Acid (μg/mL)	35.6	38.1	38.6	33.5	3.07	0.650
Total VFAs (mM)	92.9	101	90.8	104	5.38	0.222
Fatty Acids (%)						
Acetate	46.1[a]	46.5[a]	45.4[a]	43.0[b]	0.72	0.004
Propionate	37.8	36.0	38.0	40.2	1.09	0.054
Butyrate	10.1	11.1	10.2	10.6	0.71	0.761
Valerate	4.62	4.87	4.48	5.13	0.43	0.716
BCVFA	1.40[ab]	1.62[a]	1.78[a]	1.15[b]	0.17	0.048
A:P	1.26[a]	1.34[a]	1.23[ab]	1.09[b]	0.05	0.010
Microbial population						
Total Bacteria	9.27	9.35	9.32	9.26	0.04	0.273
Archaea	5.65	5.63	5.60	5.47	0.07	0.260
Protozoa	nd	nd	nd	nd		
Fungi	nd	nd	nd	nd		

Abbreviations: VFAs = Volatile Fatty Acids; BCVFA = branched-chain; A:P = Acetate:Propionate ratio, nd: non-detectable.
[1] CTL: Control Diet with no additives.
[2] EO: Essential Oil Diet containing Digestarom from DSM Nutritional Products, Switzerland.
[3] SYN: Synbiotic Diet containing YANG and Levucell from Lallemand Inc. Animal Nutrition, Canada.
[4] MIX Mixed Diet a combination of probiotics, yeast fractions, oregano-based essential oil, and sodium butyrate.
a, b Values within a row with different superscripts differ significantly at $P < 0.05$.

Table 4
Effect of feed additive nutritional intervention on rumen fermentation profile and microbial biomass (Log_{10} Copy/mL) at 2 months postweaning.

Parameter	Treatment				SEM	P-value
	CTL[1]	EO[2]	SYN[3]	MIX[4]		
pH	6.83[a]	6.61[ab]	6.41[b]	6.42[b]	0.11	0.019
Lactic Acid (μg/mL)	40.3	41.8	41.8	41.2	3.15	0.984
Total VFAs (mM)	76.0[b]	102[a]	94.4[a]	93.8[a]	4.72	0.002
Fatty Acids (%)						
Acetate	44.8	44.7	45.0	44.7	0.64	0.984
Propionate	42.9	41.4	42.7	41.6	0.81	0.414
Butyrate	7.50[b]	9.31[a]	8.28[ab]	9.08[a]	0.50	0.048
Valerate	3.33	3.22	3.04	3.55	0.22	0.460
BCVFA	1.47[a]	1.33[ab]	0.91[c]	1.08[bc]	0.10	0.001
A:P	1.05	1.11	1.07	1.10	0.04	0.710
Microbial population						
Total Bacteria	10.5	10.5	10.6	10.6	0.31	0.997
Archaea	6.33	6.43	6.34	6.39	0.17	0.973
Protozoa	nd	nd	nd	nd		
Fungi	3.20	3.19	3.14	3.22	0.04	0.387

Abbreviations: VFAs = Volatile Fatty Acids; BCVFA = branched-chain; A:P = Acetate:Propionate ratio, nd: non-detectable.
[1] CTL: Control Diet with no additives.
[2] EO: Essential Oil Diet containing Digestarom from DSM Nutritional Products, Switzerland.
[3] SYN: Synbiotic Diet containing YANG and Levucell from Lallemand Inc. Animal Nutrition, Canada.
[4] MIX Mixed Diet a combination of probiotics, yeast fractions, oregano-based essential oil, and sodium butyrate.
a,b,c Values within a row with different superscripts differ significantly at $P < 0.05$.

Table 5
Effect of nutritional intervention on body weight gain.

Parameter	Treatment				SEM	P-value
	CTL[1]	EO[2]	SYN[3]	MIX[4]		
Weight 1 (kg)	57.3	57.9	58.5	57.7	0.83	0.775
Weight 2 (kg)	93.2	91.2	90.3	93.9	2.00	0.553
Weight 3 (kg)	161	159	159	162	3.75	0.887
ADG 1 (g/day)	781	723	692	786	35.7	0.190
ADG 2 (g/day)	1169	1188	1187	1175	45.2	0.989
TADG (g/day)	998	980	969	1003	33.9	0.889

Abbreviations: ADG = Average Daily Gain. TADG = Total Average Daily Gain.
[1] CTL: Control Diet with no additives.
[2] EO: Essential Oil Diet containing Digestarom from DSM Nutritional Products, Switzerland.
[3] SYN: Synbiotic Diet containing YANG and Levucell from Lallemand Inc. Animal Nutrition, Canada.
[4] MIX Mixed Diet a combination of probiotics, yeast fractions, oregano-based essential oil, and sodium butyrate.

4. Discussion

4.1. Blood parameters

Before weaning, greater values for CK and GOT were observed with the MIX and CTL groups. Although this may indicate an enhanced metabolic activity which could increase the load on liver and muscle cells, values for all treatments were within the normal physiological ranges (Kaneko et al., 2008). The significantly higher levels of BHB in EO and MIX treatments suggest enhanced microbial activity in the rumen by the experimental treatments, since butyrate is transformed into BHB in the rumen wall during absorption (Belanche et al., 2020). This is further discussed below in relation to rumen development.

During the postweaning period, the serum cortisol levels were greater than at weaning, which is the result of the physiological increase as the animals grow and mature (Biriukova et al., 2023). Values were greater in CTL animals than in the other three experimental groups. Weaning is one of the more challenging and stressful events in dairy calves. If managed poorly, weaning stress can adversely affect several growth and health indicators, such as feed intake, ADG, as well as indicators of stress response and negative energy balance (McCoard et al., 2019). Since all calves went through the same weaning process, it can be hypothesized that the effects of feed additives treatments applied to calves alleviated the stress caused by transitioning from liquid to solid feed regime (Agustinho et al., 2024).

The main parameter that showed a consistent effect across all feed additives treatments compared to CTL is the elevated concentration of BHB. As stated above, BHB concentrations in blood can be used as indictor of rumen fermentative functional development. Agustinho et al. (2024) and Wolfe et al. (2023), demonstrated that animals that are gradually weaned had a greater grain intake than those weaned abruptly, which is the main factor explaining elevated serum BHB concentration. Also, rumen butyrate is well

known for playing an important role in the rumen papillae development (Sander et al., 1959). Our results suggest improved rumen fermentation in treated animals two months postweaning and potentially better rumen capacity for handling solid diet fermentation.

Animals that received the MIX treatment had significantly lower levels of glucose, which coincided with elevated insulin levels (Dimitriadis et al., 2021). The reason for such effect, considering that all animas received the same diet, are not clear.

4.2. Rumen fermentation and quantitative PCR

4.2.1. Pre- vs. postweaning

The results of the rumen fermentation profile suggested a well-established solid feed consumption and therefore rumen microbial fermentation activity at weaning since total VFA concentrations were very similar between samples collected at weaning and two months postweaning. The values were within the range reported for dairy calves between 50 and 80 days of life (80–138 mM, Hao et al., 2021; Pazoki et al., 2017) and indicate that animals were ready for weaning. Likewise, rumen pH values were within the physiological range for optimal microbial activity (6.1–6.6, Therion et al., 1982) and they were lower in animals at weaning than postweaning, which could be explained by lower forage consumption in younger animals and therefore reduced rumination and salivation activity (Rahimi et al., 2023).

Despite similar overall fermentation values between weaning and postweaning, the concentrations of bacteria, archaea and fungi were increased after weaning (average across groups 9.34–10.6 for bacteria, 5.59–6.37 for archaea, 0-3.19 for fungi, Log10 Copy numbers/mL) and no protozoa were detected in animals at weaning and postweaning. Different works in the literature described the microbial community successions that occur in the rumen from birth to weaning and after, when animals receive exclusively solid feeds (Malmuthuge et al., 2015; Yáñez-Ruiz et al., 2015; Meale et al., 2017). Some of the functional populations, as well as taxa present in the rumen of adult animals, appear very early after birth and establish in a progressive way and in a defined sequence (Morgavi et al., 2020). In agreement with our results, prokaryotes, bacteria and archaea, are the first colonizers of the rumen and are more abundant as the rumen develops (Abecia et al., 2014b) and ukaryotes (fungi and protozoa) take longer to colonize the rumen and reach lower abundances (Morgavi et al., 2010). Although anaerobic fungi can be enumerated in the rumen of lambs by 8–10 days after birth, their lower metabolic rate and primarily cellulolytic activity make their establishment in the rumen to occur later (Fonty et al., 1987). Our results about the lack of anaerobic fungi in most animals agree with those from Huuki et al. (2022) who, in a trial conducted to test the impact of early life inoculation with rumen fluid from adult animals to dairy calves, could only detect fungi in the rumen of one control animal and 2 in the treated ones at 2 months of age. By following rumen microbial community development postweaning, Huuki et al. (2022) demonstrated that the quantity of rumen fungi remained low until month 4, and that the development of the anaerobic fungi community structure and the increase in richness was gradual and continued at least until month 10, after which it stabilized.

Unlike bacteria, archaea and fungi, protozoa do not establish when new-borns are isolated from their dams and other adult animals shortly after birth as adult animals are the only source of protozoal inoculation (Abecia et al., 2014a; Fonty et al., 1988). In addition, ciliate protozoa require the presence of a complex microbiota to establish (Fonty et al., 1983, 1988). On the basis of that, and in agreement with recent works (Belanche et al., 2019; Huuki et al., 2022) it is very unlikely that protozoa colonized the rumen of the calves in this study.

4.3. Inclusion of additives

With regards to the impact of the feed additives experimental treatments, it is important to distinguish between effects at weaning (until when the three different treatments were applied) and two months after weaning (when animals had been fed the same MIX diet and therefore only persistency effects could be assessed). At weaning, the impact of feed additives treatments on rumen fermentation parameters was minimal, with the exception of lowered acetate molar proportion, a tendency to increase propionate and subsequently reduced the acetate/propionate ratio in animals receiving the MIX treatment. The effect on propionate proportion in the rumen is consistent with the tendency to increase glucose levels in the blood in the same animals. In addition to gluconeogenesis, the glucose circulating in the bloodstream can have two main sources: lactose absorbed from milk replacer (Welboren et al., 2021) and propionate production in the rumen (Wiltrout and Satter, 1972). Since the milk replacer allowance was the same across animals because it was provided individually, the increase in propionate production in the rumen is the most likely reason to explain the tendency to have greater glucose levels in blood in group MIX. The MIX treatment contained yeast probiotics (which was present in SYN treatment), butyric acid and oregano plant extract. With such complex combination, it is not possible to assign an effect on a single component. Oregano extracts or leaves contain essential oils, such as carvacrol and thymol, and oregano extracts supplementation has been studied in dairy cows (Kolling et al., 2018; Stivanin et al., 2019; Benchaar, 2020; Vizzotto et al., 2021) and dairy calves (de Paris et al., 2020; Heisler et al., 2020), showing positive effects on feeding behaviour (Stivanin et al., 2019; Heisler et al., 2020), reducing methane emission (Kolling et al., 2018) and improving redox status of calves (de Paris et al., 2020) and cows (Vizzotto et al., 2021). However, the effect on preweaned calves is not well known. Recently, Ritt et al. (2023) showed that supplying oregano extract to preweaned dairy calves increased the diversity of bacterial population in the rumen and jejunum, affecting both gram-positive and negative bacteria and decreasing the abundance of potential pathogenic bacteria, while improving apparent digestibility. It is likely that the supplementation with oregano extract in our trial enhanced the development of propionic producing bacteria, which would need to be confirmed by sequencing analyses. The other component of the MIX treatment that was not included in either SYN or EO is butyric acid. Besides serving as an energy substrate, butyrate is an important stimulator and regulator of the ruminal epithelium growth and function (Penner et al., 2011). In pre-ruminant calves, infusion of butyrate directly into the lumen of the developing rumen stimulated

ruminal epithelial cells proliferation and reduced their apoptosis (Sakata and Tamate, 1978; Mentschel et al., 2001). This resulted in longer rumen papillae (Mentschel et al., 2001) and, in consequence, most likely a larger surface area for nutrient absorption (Malhi et al., 2013; Shen et al., 2005). The stimulatory effect of butyrate on the ruminal epithelium growth has been known for years and promotion of its formation in the rumen, by feeding diets high in starch and sugars, is widely used as a means of accelerating forestomach development in calves (Heinrichs, 2005; Lesmeister and Heinrichs, 2005). An enhanced development of the papillae in the rumen of MIX calves could have also contributed to the increased glucose levels in blood from better absorption of microbial fermentation propionate.

The analyses of samples 2 months postweaning showed a significant effect of treatments on rumen pH, total VFA and BCFA. The lowered pH values and greater total VFA concentrations in the three treatments compared to CTL are a good indication of enhanced overall microbial fermentation. The pH values were within the physiological range for optimal microbial fermentation and did not show any risk of acidosis, which is a common concerned in the intensive beef production systems (Devant and Marti, 2020). The positive impact of providing a range of additives to young ruminants during the rumen development stages on rumen fermentation has been described by many authors and in particular with regards to the treatments used in this trial. For example the use of yeast probiotics and their products (Alugongo et al., 2017; Chaucheyras-Durand et al., 2019), butyrate (Górka et al., 2018), and essential oils (Santos et al., 2015). More recently, the benefits of combining additives with different modes of action or microbial targets have been investigated (Salazar et al., 2019; Stefańska et al., 2021), reporting beneficial synergistic effects for both animal health and performance.

However, what is not yet fully elucidated or less clear is the persistency of the effects later in life of the animals (Yáñez-Ruiz et al., 2015) once the treatments have ceased. In this study, the CTL group did not receive any treatment during the first 2 months of life until weaning, and then animals received the same treatment as the other three groups (MIX). The reason for that feeding management is that the MIX diet is the one used in the commercial farm where the trial was conducted. Our results show that regardless the treatment received that can enhance a positive rumen development, the treatment needs to be applied during the preweaning period, otherwise the positive effect is not achieved, suggesting that the first 2 months of life is a critical window for development and future functioning of rumen activity. The literature on medium to long-term effects of interventions applied preweaning is very scarce and focus on specific topics (i.e. methane emissions), and limited types of interventions (direct rumen fluid inoculation) (Abecia et al., 2013; De Barbieri et al., 2015; Meale et al., 2021). Recently, Huuki et al. (2022) demonstrated using twin dairy calves that oral administration of rumen fluid until weaning induced transient changes in early rumen microbiome maturation and later production performance, although the mechanisms that mediate these effects were not clarified. Although supplying live rumen microorganisms is not a feasible practice in commercial farms, the results showed potential for 'programming' the rumen microbial ecosystem in early-life. Since no differences have been observed in the abundance of bacteria or archaea, subsequent sequencing analyses are required to attribute the persistency of differences in rumen fermentation profile to microbial diversity and function.

4.4. Body weight gain

The lack of effect of the treatments on body weight, despite the positive impact on rumen fermentation, has been largely described in previous works conducted with pre-ruminants supplied with different type of feed additives (Belanche et al., 2020; Greenwood et al., 2024; Reddy et al., 2020; Stefańska et al., 2021). These and other works highlighted the relevance of optimizing rumen functional development around weaning for two main reasons: i) facing potential environmental challenges (i.e. weather conditions, pathogens) and ii) performing better in terms of body weight gain during the growing and finishing periods. In this study, the management and raising conditions of the calves after arrival to the farm were likely not challenging enough to represent and advantage for the treated calves. However, the greater homogeneity (lower standard deviation) of the weights in treated animals at weaning and after weaning represents an advantage for the overall farm management and planning, meaning most animals can reach the final stage before marketing in a shorter and similar period of time. This is rather beneficial in contrast to having rapidly growing animals and not so fast-growing ones in the same lot.

5. Conclusions

Under the commercial conditions of this study, the inclusion in the starter concentrate of feed additives based on essential oils and yeast probiotics, enhanced rumen fermentation development; however, the treatment needs to be applied during the preweaning period, otherwise the positive effect would not be achieved, suggesting that the first 2 months of life are a critical window for development and future functioning of rumen activity. The positive effects on rumen functional development did not translate into productivity gains but better uniformity of the weights in the animal's groups, which can be beneficial for management and planning in the farm.

CRediT authorship contribution statement

E. Romera-Recio: Writing – original draft, Methodology, Formal analysis, Data curation. E. Ramos-Morales: Writing – review & editing, Supervision, Data curation. A. Belanche: Supervision, Methodology, Funding acquisition, Conceptualization. M. Hassan: Methodology. P. Romero: Methodology. A. Gómez: Methodology. I. Rivelli: Writing – review & editing, Methodology. N. Llanes: Validation, Methodology, Investigation, Conceptualization. J. Torra: Validation, Data curation, Conceptualization. D.R. Yáñez-Ruiz: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology,

Investigation, Conceptualization.

Funding

This study has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 101000213 (HoloRuminant). Alejandro Belanche was supported by the Spanish State Research Agency (AEI) through a Ramon y Cajal research contract RYC2019-027764-I, project PID2021-123206OB-100.

Author statement

I am writing to submit the revised version of our manuscript entitled, "Effects of feed additives in male dairy beef calves on physiological status and rumen microbial fermentation pre- and postweaning" to be considered for publication. We would like to thank the reviewer's comments and suggestions, which certainly help to improve the quality of the manuscript. All of them have been incorporated into the revised manuscript.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use any AI or AI-assisted technologies in the preparation of this work.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Acknowledgements

The authors wish to thank Isabel Jiménez, Elisabeth Jiménez and Tamara García for their assistance in samples collection and analyses, to Cooperative d'Ivars in Ivars d'Urgell for kindly providing access to their facilities and collection of biological samples from animals and to Lallemand Animal Nutrition for supplying yeast-based probiotics feed additives.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.anifeedsci.2025.116243.

References

Abecia, L., Martín-García, A.I., Martinez, G., Newbold, C.J., Yáñez-Ruiz, D.R., 2013. Nutritional intervention in early life to manipulate rumen microbial colonization and methane output by kid goats postweaning. J. Anim. Sci. 91 (10), 4832–4840. https://doi.org/10.2527/JAS.2012-6142.

Abecia, L., Waddams, K.E., Martínez-Fernandez, G., Martín-García, A.I., Ramos-Morales, E., Newbold, C.J., Yáñez-Ruiz, D.R., 2014b. An antimethanogenic nutritional intervention in early life of ruminants modifies ruminal colonization by archaea. Archaea 2014. https://doi.org/10.1155/2014/841463.

Abecia, L., Ramos-Morales, E., Martínez-Fernandez, G., Arco, A., Martín-García, A.I., Newbold, C.J., Yáñez-Ruiz, D.R., 2014a. Feeding management in early life influences microbial colonisation and fermentation in the rumen of newborn goat kids. Anim. Prod. Sci. 54 (9), 1449–1454. https://doi.org/10.1071/AN14337.

Agustinho, B.C., Wolfe, A., Tsai, C.Y., Pereira, L.M., Konetchy, D.E., Laarman, A.H., Rezamand, P., 2024. Effects of weaning age and pace on blood metabolites, cortisol concentration, and mRNA abundance of inflammation-related genes in gastrointestinal, adipose, and liver tissue of Holstein dairy calves. J. Dairy Sci. 107 (6), 3988–3999. https://doi.org/10.3168/JDS.2023-23642.

Alugongo, G.M., Xiao, J.X., Chung, Y.H., Dong, S.Z., Li, S.L., Yoon, I., Wu, Z.H., Cao, Z.J., 2017. Effects of Saccharomyces cerevisiae fermentation products on dairy calves: Performance and health. J. Dairy Sci. 100 (2), 1189–1199. https://doi.org/10.3168/JDS.2016-11399.

AOAC International, 2005. Official methods of analysis of AOAC International.

Arco-Pérez, A., Ramos-Morales, E., Yáñez-Ruiz, D.R., Abecia, L., Martín-García, A.I., 2017. Nutritive evaluation and milk quality of including of tomato or olive by-products silages with sunflower oil in the diet of dairy goats. Anim. Feed Sci. Technol. 232, 57–70. https://doi.org/10.1016/j.anifeedsci.2017.08.008.

Baldwin, R.L., McLeod, K.R., Klotz, J.L., Heitmann, R.N., 2004. Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. J. Dairy Sci. 87, 55–65. https://doi.org/10.3168/jds.S0022-0302(04)70061-2.

Belanche, A., Yáñez-Ruiz, D.R., Detheridge, A.P., Griffith, G.W., Kingston-Smith, A.H., Newbold, C.J., 2019. Maternal versus artificial rearing shapes the rumen microbiome having minor long-term physiological implications. Environ. Microbiol. 21 (11), 4360–4377. https://doi.org/10.1111/1462-2920.14801.

Belanche, A., Palma-Hidalgo, J.M., Nejjam, I., Jiménez, E., Martín-García, A.I., Yáñez-Ruiz, D.R., 2020. Inoculation with rumen fluid in early life as a strategy to optimize the weaning process in intensive dairy goat systems. J. Dairy Sci. 103 (6), 5047–5060. https://doi.org/10.3168/jds.2019-18002.

Benchaar, C., 2020. Feeding oregano oil and its main component carvacrol does not affect ruminal fermentation, nutrient utilization, methane emissions, milk production, or milk fatty acid composition of dairy cows. J. Dairy Sci. 103 (2), 1516–1527. https://doi.org/10.3168/JDS.2019-17230.

Biriukova, O., Lesniak, Yu, Basovskiy, D., & Vintoniv, N., 2023. Dynamics of the cortisol level in the blood of young cattle, 25–30. https://doi.org/10.61562/MGFA2023.03.

Brunel, Stéfano, Dallago, Melgaço, Almeida, Bezerra de, Assis, Zorzan de, De Bento Calzada, Braga Brasileiro, de Alvarenga, Morato, Barbosa, Rodrigues, McManus, Diaz, Broom, Moreno, 2018. Hemato-biochemical profile of meat cattle submitted to different types of pre-loading handling and transport times. Int. J. Vet. Sci. Med. 6 (1), 90–96. https://doi.org/10.1016/j.ijvsm.2018.04.002.

Chaucheyras-Durand, F., Ameilbonne, A., Auffret, P., Bernard, M., Mialon, M.M., Dunière, L., Forano, E., 2019. Supplementation of live yeast based feed additive in early life promotes rumen microbial colonization and fibrolytic potential in lambs. Sci. Rep. 2019 9 (1), 1–16. https://doi.org/10.1038/s41598-019-55825-0.

De Barbieri, I., Hegarty, R.S., Silveira, C., Oddy, V.H., 2015. Positive consequences of maternal diet and post-natal rumen inoculation on rumen function and animal performance of Merino lambs. Small Rumin. Res. 129, 37–47. https://doi.org/10.1016/J.SMALLRUMRES.2015.05.017.
Devant, M., Marti, S., 2020. Strategies for feeding unweaned dairy beef cattle to improve their health. Animals 10 (10), 1–20. https://doi.org/10.3390/ANI10101908.
Diao, Q., Zhang, R., Fu, T., 2019. Review of strategies to promote Rumen development in calves. Animals 9 (8). https://doi.org/10.3390/ANI9080490.
Dimitriadis, G.D., Maratou, E., Kountouri, A., Board, M., Lambadiari, V., 2021. Regulation of postabsorptive and postprandial glucose metabolism by insulin-dependent and insulin-independent mechanisms: an integrative approach. Nutrients 13 (1), 1–33. https://doi.org/10.3390/NU13010159.
Eurostat, 2022. Agricultural production - livestock and meat. Data extracted in September 2023. Retrieved July 31, 2024, from ⟨https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Agricultural_production_-_livestock_and_meat⟩.
Fonty, G., Gouet, P., Jouany, J.P., Senaud, J., 1983. Ecological factors determining establishment of cellulolytic bacteria and protozoa in the rumens of meroxenic lambs. J. Gen. Microbiol. 129 (1), 213–223. https://doi.org/10.1099/00221287-129-1-213.
Fonty, G., Gouet, P., Jouany, J.P., Senaud, J., 1987. Establishment of the microflora and anaerobic fungi in the rumen of lambs. J. Gen. Microbiol. 133 (7), 1835–1843. https://doi.org/10.1099/00221287-133-7-1835.
Fonty, G., Senaud, J., Jouany, J.P., Gouet, P., 1988. Establishment of ciliate protozoa in the rumen of conventional and conventionalized lambs: influence of diet and management conditions. Can. J. Microbiol. 34 (3), 235–241. https://doi.org/10.1139/M88-044.
Górka, P., Kowalski, Z.M., Zabielski, R., Guilloteau, P., 2018. Invited review: Use of butyrate to promote gastrointestinal tract development in calves. J. Dairy Sci. 101 (6), 4785–4800. https://doi.org/10.3168/JDS.2017-14086.
Greenwood, E.C., Torok, V.A., Agenbag, B., Hynd, P.I., 2024. Manipulation of neonatal ruminal populations at birth results in sustained effects on microbial populations and measures of health and production in merino and suffolk lambs. Livest. Sci. 280, 105406. https://doi.org/10.1016/J.LIVSCI.2024.105406.
Hao, Y., Gong, Y., Huang, S., Ji, S., Wang, W., Wang, Y., Yang, H., Cao, Z., Li, S., 2021. Effects of age, diet CP, NDF, EE, and starch on the rumen bacteria community and function in dairy cattle. Microorganisms 9 (8). https://doi.org/10.3390/MICROORGANISMS9081788.
Heinrichs, J., 2005. Rumen development in the dairy calf. Adv. Dairy Technol. 17, 179–187.
Heisler, G., Fischer, V., de Paris, M., Veber Angelo, I.D., Panazzolo, D.M., Zanela, M.B., 2020. Effect of green tea and oregano extracts fed to preweaned Jersey calves on behavior and health status. J. Vet. Behav. 37, 36–40. https://doi.org/10.1016/J.JVEB.2020.03.002.
Huuki, H., Tapio, M., Mäntysaari, P., Negussie, E., Ahvenjärvi, S., Vilkki, J., Vanhatalo, A., Tapio, I., 2022. Long-term effects of early-life rumen microbiota modulation on dairy cow production performance and methane emissions. Front. Microbiol. 13, 983823. https://doi.org/10.3389/FMICB.2022.983823/BIBTEX.
Kaneko, J.J., Harvey, J.W., Bruss, M.L., 2008. Clinical Biochemistry of Domestic Animals, Sixth Edition. Clinical Biochemistry of Domestic Animals, Sixth Edition, 1–916. https://doi.org/10.1016/B978-0-12-370491-7.X0001-3.
Kolling, G.J., Stivanin, S.C.B., Gabbi, A.M., Machado, F.S., Ferreira, A.L., Campos, M.M., Tomich, T.R., Cunha, C.S., Dill, S.W., Pereira, L.G.R., Fischer, V., 2018. Performance and methane emissions in dairy cows fed oregano and green tea extracts as feed additives. J. Dairy Sci. 101 (5), 4221–4234. https://doi.org/10.3168/jds.2017-13841.
Lesmeister, K.E., Heinrichs, A.J., 2005. Effects of adding extra molasses to a texturized calf starter on rumen development, growth characteristics, and blood parameters in neonatal dairy calves. J. Dairy Sci. 88 (1), 411–418. https://doi.org/10.3168/JDS.S0022-0302(05)72702-8.
Malhi, M., Gui, H., Yao, L., Aschenbach, J.R., Gäbel, G., Shen, Z., 2013. Increased papillae growth and enhanced short-chain fatty acid absorption in the rumen of goats are associated with transient increases in cyclin D1 expression after ruminal butyrate infusion. J. Dairy Sci. 96 (12), 7603–7616. https://doi.org/10.3168/JDS.2013-6700.
Malmuthuge, N., Griebel, P.J., Guan, L.L., 2015. The gut microbiome and its potential role in the development and function of newborn calf gastrointestinal tract. Front. Vet. Sci. 2. https://doi.org/10.3389/FVETS.2015.00036.
McCoard, S., Heiser, A., Lowe, K., Molenaar, A., MacLean, P., Johnstone, P., Leath, S., Hoskin, S.O., Khan, M.A., 2019. Effect of weaning age on growth, mammary gland development, and immune function in Holstein Friesian calves fed conserved alfalfa (FiberStart). J. Dairy Sci. 102 (7), 6076–6087. https://doi.org/10.3168/JDS.2018-15615.
Meale, S.J., Chaucheyras-Durand, F., Berends, H., Guan, L.L., Steele, M.A., 2017. From pre- to postweaning: Transformation of the young calf's gastrointestinal tract. J. Dairy Sci. 100 (7), 5984–5995. https://doi.org/10.3168/JDS.2016-12474.
Meale, S.J., Popova, M., Saro, C., Martin, C., Bernard, A., Lagree, M., Yáñez-Ruiz, D.R., Boudra, H., Duval, S., Morgavi, D.P., 2021. Early life dietary intervention in dairy calves results in a long-term reduction in methane emissions. Sci. Rep. 11 (1), 1–13. https://doi.org/10.1038/s41598-021-82084-9.
Mentschel, J., Leiser, R., Mülling, C., Pfarrer, C., Claus, R., 2001. Butyric acid stimulates rumen mucosa development in the calf mainly by a reduction of apoptosis. Arch. Anim. Nutr. 55 (2), 85–102. https://doi.org/10.1080/17450390109386185.
Morgavi, D.P., Forano, E., Martin, C., Newbold, C.J., 2010. Microbial ecosystem and methanogenesis in ruminants. Animal 4 (7), 1024–1036. https://doi.org/10.1017/S1751731110000546.
Morgavi, D.P., Popova, M., Yañez-Ruiz, D.R., Forano, E., 2020. Colonization and establishment of the rumen microbiota - opportunities to influence productivity and methane emissions. In: McSweeney, In.C., Mackie, R. (Eds.), Improving Rumen Function (1st Edition). Burleigh Dodds Science Publishing, pp. 3–28. https://doi.org/10.1201/9781003047841-1.
Palma-Hidalgo, J.M., Yáñez-Ruiz, D.R., Jiménez, E., Martín-García, A.I., Belanche, A., 2021. Presence of adult companion goats favors the rumen microbial and functional development in artificially reared kids. Front. Vet. Sci. 8. https://doi.org/10.3389/fvets.2021.706592.
de Paris, M., Stivanin, S.C.B., Klein, C.P., Vizzotto, E.F., Passos, L.T., Angelo, I.D.V., Zanela, M.B., Stone, V., Matté, C., Heisler, G., Fischer, V., 2020. Calves fed with milk from cows receiving plant extracts improved redox status. Livest. Sci. 242, 104272. https://doi.org/10.1016/J.LIVSCI.2020.104272.
Pazoki, A., Ghorbani, G.R., Kargar, S., Sadeghi-Sefidmazgi, A., Drackley, J.K., Ghaffari, M.H., 2017. Growth performance, nutrient digestibility, ruminal fermentation, and rumen development of calves during transition from liquid to solid feed: effects of physical form of starter feed and forage provision. Anim. Feed Sci. Technol. 234, 173–185. https://doi.org/10.1016/J.ANIFEEDSCI.2017.06.004.
Penner, G.B., Steele, M.A., Aschenbach, J.R., McBride, B.W., 2011. RUMINANT NUTRITION SYMPOSIUM: Molecular adaptation of ruminal epithelia to highly fermentable diets. J. Anim. Sci. 89 (4), 1108–1119. https://doi.org/10.2527/JAS.2010-3378.
Rahimi, A., Ghorbani, G.R., Hashemzadeh, F., Mirzaei, M., Saberipour, H.R., Ahmadi, F., Ghaffari, M.H., 2023. Impact of corn processing and weaning age on calf performance, blood metabolites, rumen fermentation, and behavior. Sci. Rep. 2023 13 (1), 1–13. https://doi.org/10.1038/s41598-023-37728-3.
Ramos-Morales, E., Arco-Pérez, A., Martín-García, A.I., Yáñez-Ruiz, D.R., Frutos, P., Hervás, G., 2014. Use of stomach tubing as an alternative to rumen cannulation to study ruminal fermentation and microbiota in sheep and goats. Anim. Feed Sci. Technol. 198, 57–66. https://doi.org/10.1016/J.ANIFEEDSCI.2014.09.016.
Reddy, P.R.K., Elghandour, M.M.M.Y., Salem, A.Z.M., Yasaswini, D., Reddy, P.P.R., Reddy, A.N., Hyder, I., 2020. Plant secondary metabolites as feed additives in calves for antimicrobial stewardship. Anim. Feed Sci. Technol. 264, 114469. https://doi.org/10.1016/J.ANIFEEDSCI.2020.114469.
Rey, M., Enjalbert, F., Combes, S., Cauquil, L., Bouchez, O., Monteils, V., 2014. Establishment of ruminal bacterial community in dairy calves from birth to weaning is sequential. J. Appl. Microbiol. 116 (2), 245–257. https://doi.org/10.1111/JAM.12405.
Ritt, L.A., Orso, C., Silveira, A.K., Frazzon, J., de Vargas, D.P., Wagner, R., de Oliveira, F.C., Nörnberg, J.L., Fischer, V., 2023. Oregano extract fed to pre-weaned dairy calves. Part 1: Effects on intake, digestibility, body weight, and rumen and intestinal bacteria microbiota. Livest. Sci. 269. https://doi.org/10.1016/J.LIVSCI.2023.105165.
Sakata, T., Tamate, H., 1978. Rumen epithelial cell proliferation accelerated by rapid increase in intraruminal butyrate. J. Dairy Sci. 61 (8), 1109–1113. https://doi.org/10.3168/jds.S0022-0302(78)83694-7.
Salazar, L.F.L., Nero, L.A., Campos-Galvão, M.E.M., Cortinhas, C.S., Acedo, T.S., Tamassia, L.F.M., Busato, K.C., Morais, V.C., Rotta, P.P., Silva, A.L., Marcondes, M.I., 2019. Effect of selected feed additives to improve growth and health of dairy calves. PLOS ONE 14 (5), e0216066. https://doi.org/10.1371/JOURNAL.PONE.0216066.
Sander, E.G., Warner, R.G., Harrison, H.N., Loosli, J.K., 1959. The stimulatory effect of sodium butyrate and sodium propionate on the development of rumen mucosa in the young calf. J. Dairy Sci. 42 (9), 1600–1605. https://doi.org/10.3168/JDS.S0022-0302(59)90772-6.

Santos, F.H.R., De Paula, M.R., Lezier, D., Silva, J.T., Santos, G., Bittar, C.M.M., 2015. Essential oils for dairy calves: effects on performance, scours, rumen fermentation and intestinal fauna. Animal 9 (6), 958–965. https://doi.org/10.1017/S175173111500018X.

Shen, Z., Kuhla, S., Zitnan, R., Seyfert, H.M., Schneider, F., Hagemeister, H., Chudy, A., Löhrke, B., Blum, J.W., Hammon, H.M., Voigt, J., 2005. Intraruminal infusion of n-butyric acid induces an increase of ruminal papillae size independent of IGF-1 system in castrated bulls. Arch. Anim. Nutr. 59 (4), 213–225. https://doi.org/10.1080/17450390500216894.

Stefańska, B., Sroka, J., Katzer, F., Goliński, P., Nowak, W., 2021. The effect of probiotics, phytobiotics and their combination as feed additives in the diet of dairy calves on performance, rumen fermentation and blood metabolites during the preweaning period. Anim. Feed Sci. Technol. 272, 114738. https://doi.org/10.1016/J.ANIFEEDSCI.2020.114738.

Stivanin, S.C.B., Vizzotto, E.F., de Paris, M., Zanela, M.B., Passos, L.T., Veber Angelo, I.D., Fischer, V., 2019. Addition of oregano or green tea extracts into the diet for Jersey cows in transition period. Feeding and social behavior, intake and health status. Plant extracts for cows in the transition period. Anim. Feed Sci. Technol. 257, 114265. https://doi.org/10.1016/J.ANIFEEDSCI.2019.114265.

Therion, J.J., Kistner, A., Kornelius, J.H., 1982. Effect of pH on growth rates of rumen amylolytic and lactilytic bacteria. Appl. Environ. Microbiol. 44 (2), 428–434. https://doi.org/10.1128/AEM.44.2.428-434.1982.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74 (10), 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Vizzotto, E.F., Stivanin, S.C.B., de Paris, M., Passos, L.T., Werncke, D., Klein, C.P., Stone, V., Matté, C., Zanela, M.B., Fischer, V., 2021. Supplementation with green tea and oregano extracts on productive characteristics, blood metabolites, and antioxidant status of Jersey cows during the transition period. Animal 15 (2), 100032. https://doi.org/10.1016/J.ANIMAL.2020.100032.

Welboren, A.C., Hatew, B., López-Campos, O., Cant, J.P., Leal, L.N., Martín-Tereso, J., Steele, M.A., 2021. Effects of energy source in milk replacer on glucose metabolism of neonatal dairy calves. J. Dairy Sci. 104 (4), 5009–5020. https://doi.org/10.3168/JDS.2020-19405.

Wiltrout, D.W., Satter, L.D., 1972. Contribution of propionate to glucose synthesis in the lactating and nonlactating cow. J. Dairy Sci. 55 (3), 307–317. https://doi.org/10.3168/JDS.S0022-0302(72)85487-0.

Wolfe, A.R., Rezamand, P., Agustinho, B.C., Konetchy, D.E., Laarman, A.H., 2023. Effects of weaning strategies on health, hematology, and productivity in Holstein dairy calves. J. Dairy Sci. 106 (10), 7008–7019. https://doi.org/10.3168/JDS.2022-22738.

Yáñez-Ruiz, D.R., Abecia, L., Newbold, C.J., 2015. Manipulating rumen microbiome and fermentation through interventions during early life: a review. Front. Microbiol. 6, 162946. https://doi.org/10.3389/FMICB.2015.01133/BIBTEX.

Yeoman, C.J., Ishaq, S.L., Bichi, E., Olivo, S.K., Lowe, J., Aldridge, B.M., 2018. Biogeographical differences in the influence of maternal microbial sources on the early successional development of the bovine neonatal gastrointestinal tract. Sci. Rep. 8 (1), 1–14. https://doi.org/10.1038/s41598-018-21440-8.