Effect of the administration of a blend of essential oils, bioflavonoids, and tannins to veal calves on growth performance, health status and methane emissions

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SUMMARY

The aim of the present study was to prove the efficacy of feeding a coated blend of essential oils, combined with bioflavonoids and tannins, in the solid feed of fattening veal calves on growth performance, health, and methane (CH_4) emissions.
A total of 541 Holstein Friesian intact male calves were allotted at the arrival into two groups: i) Control, standard milk replacer and solid feed (280 calves; age: 24 days; arrival weight: 50 kg); ii) Treatment, standard milk replacer + standard solid feed supplemented with 0.3 g/100 kg live weight of the coated blend of natural compounds (261 calves; age: 24.5 days; arrival weight: 51 kg). The growth performances, such as average daily gain (ADG), final weights, solid feed intake (FI), milk replacer intake, feed conversion rate (FCR) and carcass weights, were evaluated, as well as the mortality rate and the incidence of diseases. The emissions of CH_4 were monitored continuously, using two Cynomys devices.
The Treatment have led to a significant improvement of the ADG (1.116 *vs* 1.055 kg/head/d in the Control group) (P=0.005). Consequently, the final (266.5 *vs* 255.7 kg live weights in the Control group) (P=0.0096) and carcass weights (137.9 *vs* 133.1 kg in the Control group) (P=0.034) were improved in treated animals. The total (333.48 *vs* 310.75 kg of solid feed per cycle in the Control group) (P<0.0001) and daily (1.697 *vs* 1.587 kg/head/day in the Control group) (P<0.0001) solid feed intakes were significantly higher in treated animals, while the milk replacer was fixed in the two groups. Also, there was a tendency toward a better FCR in treated animals (2.84 *vs* 2.93 in the Control group) (P=0.08). No effects were detected in terms of mortality rate, incidence of diarrhoea and of first cases of bovine respiratory disease (BRD). However, there was a tendency toward a significant reduction in the incidence of BRD relapses in the Treatment group (3.08 *vs* 16.4 % on the total animals and 15.17 *vs* 19.71 % on the first treatment in the Control group) (P=0.053). The treatment reduced also the average CH_4 (90.97 *vs* 86.20 g/m^3 in the Control group) (P<0.0001), starting to be effective from week 8 of the trial.
In conclusion, the results of the present trial highlighted that the inclusion of a blend of essential oils, bioflavonoids, and tannins in the solid feed of white veal calves can be an effective strategy to increase the production efficiency, while tackling the use of antimicrobials, improving animal welfare, and counteracting the environmental impacts.

KEY WORDS

Veal calves, essential oils, methane emissions, welfare, sustainability.

INTRODUCTION

The European veal calves farming system needs to face important challenges, to accomplish with the increasingly stringent dictates of legislation on animal welfare, as highlighted by the latest scientific opinion of the EFSA AHAW Panel (EFSA Panel on Animal Health and Animal Welfare) (1).
Besides the prolonged diatribe related to the housing in single cage versus group pens and space allowance, nowadays a great attention is again placed on the role of different types and levels of solid feeds on animal welfare and ruminal development (2,3,4). Indeed, the newest EFSA Scientific Opinion of 2023 focused the attention to the type of feeds that should be included, specifically, the importance of a correct administration of fibre (NDF) from roughage sources as the best way to ensure a correct ruminal development, animal behaviour and health. Also, it is stated that the iron deficiency, that is usually corrected by injections, should be conversely amended by using roughage sources as solid feeds, that are richer than grains in this element (1).
The continuous increase in solid feed's administration, and particularly the latest request in terms of fibre administration to veal calves, has brought to light an issue that until now had been little considered, if not neglected: the emissions of greenhouse gases (GHGs), especially of methane (CH_4), by veal calves (5,6,7).
The livestock sector is indeed responsible of about 14.5% to the total anthropogenic GHGs emissions, with CH_4 resulting from ruminal fermentation of cattle representing the biggest share (8,9). Since the high presence in the rumen of some byproducts (H_2, CO_2) is the leading cause of CH_4 production and release, a greater attention was focused on adult animals characterized by a fully developed and functional rumen (5,8). Some studies were performed to understand the possibility to

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modulate the ruminal microflora by using different feeding strategies or feed additives during the rumen development of calves to reduce the environmental impact in the adult life (6,7,10,11). Conversely, the contribution of the white veal production system, on CH_4 emissions have been neglected till present days (5).

Increasing the solid feed and fibre administration leads to a more pronounced ruminal development, both from a physical and microbiological perspective, with thus a higher fermentation activity (6,7), that might lead to an increased emission of CH_4 from the rumen (11). In the study of Tummler et al. (2020), dairy calves fed with higher levels of solid feeds, in partial replacement of the milk replacer, were characterized by increased emission levels compared to calves fed with higher milk replacer percentages (10).

Moreover, if a higher quota of the solid feeds for veal calves is going to be represent by fibre instead of the more traditional grains, a higher production of acetate is expected, with negative drawbacks on the levels of free H_2 in the rumen, the main precursor of CH_4 (12). Indeed, a higher CH_4 production was registered in veal calves fed with higher amount of fibrous solid feeds or hay in comparison of calves fed with grains or the standard amount of fibrous solid feeds (12).

Those critical aspects pave the way for the application of possible mitigation strategies to reduce the CH_4 emissions from the veal calves' sector, that represent in Europe an average production of 6 million of calves per year, corresponding to about 1 million ton of meat (13).

To the best of our knowledge, there is only one study that directly evaluate the role of a specific feed additive on CH_4 reduction in veal calves (5). Brand et al. (2021) tested the effects of a natural product based on allicin in male veal calves fed with milk replacer, wheat straw and concentrate, for 15 weeks study period, underlining a significant reduction in the CH_4 emissions in treated animals, while the production performance was unaffected (5).

The application of natural products, such as different essential oils, tannins, bioflavonoids, and different blend of them, shown interesting results in reducing CH_4 emissions in adult cattle, while maintaining or even improving animal production efficiency and welfare (14,15). Indeed, natural extracts and plan-derived feed additives are also characterized by multiple properties that might be beneficial to promote health, welfare, and resilience of farmed animals. Antioxidant, antimicrobial and antiparasitic properties are some of the main positive attributes of natural feed additives when used in livestock. Those properties are of utmost importance in the early stages of life, to support the correct development of the immune and digestive systems, while protecting the young animals from microbial agents and other external stressors. Specifically, the effectiveness of different blends of essential oils and other natural products, such as tannins, bioflavonoids and saponins, have been tested in several studies in young calves highlighting multiple beneficial effects on performance, ruminal and gastrointestinal development, diarrhoea and respiratory issues, and overall immune functionality (16, 17, 18, 19).

Improving animal health and resistance by enhancing the immune functionality is central in the veal calves farming system, where the overall incidence of diseases and the related needs of antimicrobial treatments is often high, due to a multitude of intrinsic critical points (20). Uncorrected colostrum administration in the farm of origin, as well as transportation, mixing, adaptation to the farm management, and criticality related to the specific nutritional management, are some of the main problems. Furthermore, all these challenges occur at an age at which the calf is immature, and several physiological systems are still developing and not completely functional yet, such as the gastrointestinal tract, the thermoregulatory and the acquired immune systems (21, 22, 23). Besides the general detrimental effects on performances and farm profitability, this situation must be addressed, in the light of the spreading of the antimicrobial resistance issue and to comply with the newest rules on antimicrobial usage at the farm level (24, 25, 26).

Thus, the scientific evaluation and implementation at the farm level of innovative feed additives aimed at maximising veal calves' health, with positive results also on growth and efficiency, while curbing the production of CH_4, are crucial in the veal calves farming system.

In this light, the aim of the present trial was to prove the efficacy of feeding a coated blend of coriander seed oil, eugenol, and geranyl acetate essential oils, combined with bioflavonoids and tannins, in the solid feed of fattening veal calves on growth performance, health, and CH_4 emissions.

MATERIALS AND METHODS

This trial was set up to evaluate the efficacy of a coated blend of essential oils, from cloves (*Syzygium aromaticum*), coriander seed (*Coriandrum sativum*), and geranium (*Pelargonium cucullatum*), tannins from chestnuts (*Castanea sativa*) and bioflavonoids from olives (*Olea europea*) (relative concentrations of the active principles were: EO:CT:BF = 1:2.5:0.1) (VITa Herbs - Vetos Europe SAGL, - Cadenazzo TI, Switzerland), on growth performance, health, and CH_4 emissions in white veal calves.

Animals, housing, and experimental groups

The study was carried out in a typical intensive veal calves' unit and involved 541 Holstein Friesian intact male calves, followed since their arrival in the unit through the entire fattening of about 190 days.

All the calves were housed for the first 8 weeks of ages in single cages, and after were moved in group pens, on slatted floor, with space availability in accordance with European regulation (27). The ventilation was automatically forced, based on internal temperature and humidity.

At the arrival, all the 541 calves included in the trial were randomly allotted in two groups: i) Treatment, 261 veal calves fed with standard milk replacer + solid feed supplemented with 0.3 g/100 kg live weight of the coated blend of natural compounds (VITa Herbs - Vetos Europe Sagl - Cadenazzo TI - Switzerland), ii) Control, 280 veal calves fed with standard milk replacer + solid feed.

The tested product was included directly at the feed mill during the preparation of the solid feed.

Nutritional plans and feeding procedures

All the calves involved in the trial were fed following the same nutritional plan, with the same milk replacers and solid feed, differing only for the supplementation of the tested product in the solid feed of the Treatment group.

Table 1 - Nutritional values of the three milk replacers and of the solid feed used in the present trial.

Parameter	Milk replacer 1 (d0-28)	replacer 2 Milk(d29-d56)	Milk replacer 3 (d57-end)	Solid feed (d0-end)
Dry matter, %	90	90	90	88.5
Crude protein, % on d.m.[1]	24	22	16.2	13.2
Fats, % on d.m.	19	18	18	3.2
Cellulose, % on d.m.	0.2	0.3	0.3	8.2
Ash, % on d.m.	7.5	8	7.8	4

[1]d.m. = dry matter

During the hole fattening period three different milk replacer were used, to better satisfy the specific nutritional requirements in the different physiological phases (28) (Table 1). The characteristics of the solid feed used in the trial are also reported in Table 1.
The milk replacer was delivered two time per day in two equal meals daily at 09:00 and 17:00. The solid feed was offered in a separate feed bunk from the start of the trial.

Parameters recorded

Production performances

Individual body weight was recorded at enrolment day (d0) and before slaughter. The individual average daily gain (ADG) was then calculated from d0 to the end of the trial. The days on feed needed to reach the slaughter target weight were also evaluated.
The daily milk intake was fixed, based on the feeding plan applied in the farm and equal in the two groups. However, the total milk powder intake was calculated for the entire fattening cycle, as the sum of the daily provision of milk powder. The effective pen intake of solid fed was calculated daily. The total quantities administered and refused were weighted daily. The FCR was then calculated, comparing the total feed intake (solid feed plus milk powder) per group per cycle with the total gain of weight.
At slaughterhouse, the all the carcass weights were recorded, as well as the dressing percentages. Also, the incidence of underweight carcasses, defined as carcasses with a weight lower than 110 kg, was recorded.

Health status

The individual health status was checked twice a day by the farm veterinary and qualified animal health care staff of the farm, during the entire fattening period. Any cases of morbidity and mortality were recorded, with specific attention on the incidence of bovine respiratory disease (BRD) and diarrhoea. Sick animals were treated according to the procedures, medications, and sanitary protocols adopted by the farm veterinary staff.

Methane emissions

The emissions of CH_4 emissions were recorded in both Treatment and Control groups using two Cynomys control units (Via Palestro 10 - 16121 Genova - Italy). The Cynomys control units analysed the environment every 20 seconds giving and average value every 20 minutes for a total of 72 data for each parameter a day. All the measurements were recorded and checked for outliers and abnormal values. The daily average, and then the weekly averages of CH_4 emission were then calculated.

Statistical analysis

All the data were analysed statistically using the SAS software. Production parameters (individual weights, days on feeds, carcass weights and dressing) were evaluated using the mixed procedure of SAS. The single animal was the statistical unit for those measures. The total solid feed intake per cycle and the FCR were evaluated using the same procedure with the pen included as statistical unit. The average daily feed intake was calculated daily, and the weekly averages were then used. The weekly averages were then evaluated using a mixed model for repeated measures, that accounted for the effects of the treatment, of the week and of their interactions.
The incidence of underweight carcasses, of health issues and the mortality rates were evaluated through a chi square test (PROC FREQ).
The data related to CH_4 emissions were analysed using a mixed model for repeated measures, that accounted for the effects of the treatment, of the week and of their interactions.
Differences were considered significant at $P \leq 0.05$, while a tendency was set up at $P<0.1$.

RESULTS AND DISCUSSION

Growth and production performance

Data related to growth performance are reported in Table 2. As visible, the treatment has led to a significant increase in the ADG (1.116 vs 1.055 kg/head/d in the Control group) ($P<0.005$). On average, the ADG of the Treatment group was 60 g/head/day higher. Since the starting weight were similar, the final weight resulted to be improved by the treatment (266.5 vs 255.7 kg in the Control group). Moreover, the better results were reached in less days on feed (194.7 vs 193.2 days in the Control group) ($P<0.0001$). Those better growth performances have significantly increased the carcass weight (137.9 vs 133.1 kg in the Control group) ($P<0.05$), as reported in Table 2. Moreover, the treatment also tended to reduce the percentage of underweight carcasses (carcass weight <110 kg) (18.85 *vs* 20.38% in the Control group) ($P=0.08$).
Those results can be ascribed to the positive role of the blend of natural products tested, on ruminal health and functionality, creating thus a better environment in the developing rumen of calves. Different blend of essential oils and natural com-

Table 2 - Production performance, carcass characteristics and intake of solid feed and milk replacers in the two groups. Where possible, the LSMEANs and the standard deviation are reported.

	Treatment	Control	*P-Value*
N° of calves	261	280	-
Arrival live weight, kg	51.0±4.3	50.5±3.9	ns
Arrival age, d	24.5±8.1	24±10.2	ns
Days on feed, d	193.2±3.8	194.7±2.8	<0.0001
Final weight, kg	266.5±50.9	255.7±42.3	0.0096
ADG[1], kg/head/d	1.116±0.27	1.055±0.22	0.005
Carcass weight, kg	137.9±26.9	133.1±23.4	0.0344
Dressing %	51.66±1.5	51.95±1.2	ns
Underweight carcass[2], % (n)	18.85 (46)	20.38 (53)	0.08
Milk replacer, kg/head/cycle	274	274	-
Solid feed, kg/head/cycle	333.48±12.7	310.75±27.2	*<0.0001*
Solid feed, kg/head/d	1.697±1.0	1.587±0.9	*<0.0001*
FCR[3]	2.84±0.64	2.93±0.60	*0.085*

[1]ADG: average daily gain, kg/head/d; [2] Underweight carcasses: carcasses with a weight <110 kg; [3]FCR: feed conversion rate

pounds are reported to positively affect the ruminal microflora, in both adult and young animals (14, 29). Also, their inclusion is reported to lead to a higher production of volatile fatty acids (VFAs), especially of butyrate and propionate in the rumen of young calves (29). Liu et al. (2020) found higher levels of serum VFAs, considered as a proxy for ruminal VFAs, in calves treated for 70 days with blend of essential oils, underlining that their higher concentration in treated animals was a result of a more developed rumen (30). The higher propionate production is the image of more efficient ruminal fermentation, explained by a better and higher functionality and development of propionate-producing bacteria promoted by the essential oils (29). Moreover, a higher presence of butyrate at the ruminal level, that can be stimulated using essential oils, is reported to be a functional substrate for the development, growth, and functionality of the ruminal epithelium. It is, indeed, the main nutrients involved in the growth of ruminal papillae, that are involved in the absorption of the nutrients produced at the ruminal level. Thus, a better growth of them can lead to an increased absorption of VFAs (31). Moreover, Campolina et al. (2023), found that the inclusion of essential oils in the milk replacer of pre-weaned dairy calves for 60 days have led to a better development of the intestine and of its epithelium, mainly due to higher butyrate levels also in the gut (32). Thus, based on the way of administration and eventually on the rate of digestibility in the rumen, essential oils can lead to improvements also in that part of the gastrointestinal tract, besides their primary effects in the rumen.

A more developed rumen, with a more functional microflora and more developed absorptive epithelium, can also sustain and explain the higher solid feed intake registered in the present trial. Indeed, the average daily intake of solid feed was significantly higher in treated animals, as reported in Table 2 (1.697 *vs* 1.587 kg/head/day of solid feed) (P<0.0001). Specifically, the feed intake was comparable till week 7 of the trial and started to be significantly higher in week 8 (1.080 *vs* 0.998 kg/head/d in the Control group) (P=0.01) (Figure 1). In the end, the total intake of solid feed was significantly higher in the Treatment group (33.48 *vs* 310.75 kg in the Control group) (P<0.0001). However, there was a tendency toward a better FCR in the Treatment group, due to the higher growth performances (2.84 *vs* 2.93 in the Control group) (P=0.08).

Thus, the combination of a better anatomical and functional development of the rumen, and maybe of the gastrointestinal tract, and of a stimulation of their microflora, can contribute to the explanation of the better growth performances highlighted in the present trial.

Health status

Data related to the health status are reported in Table 3. No sig-

Table 3 - Health status in the two groups.

Group	N°	BRD[1]		Diarrhoea in the first 20 d, % (n)	Mortality, % (n)
		I° pull, % (n)	Relapse, % on total (n) and % on I° pull		
Treatment	261	85.82 (224)	13.08 (34), 15.17	27.9 (73)	6.51 (17)
Control	280	85.71 (240)	16.4 (46), 19.71	26.1 (73)	7.14 (20)
P-Value	-	*ns*	*0.0533*	*ns*	*ns*

[1]BRD= bovine respiratory disease

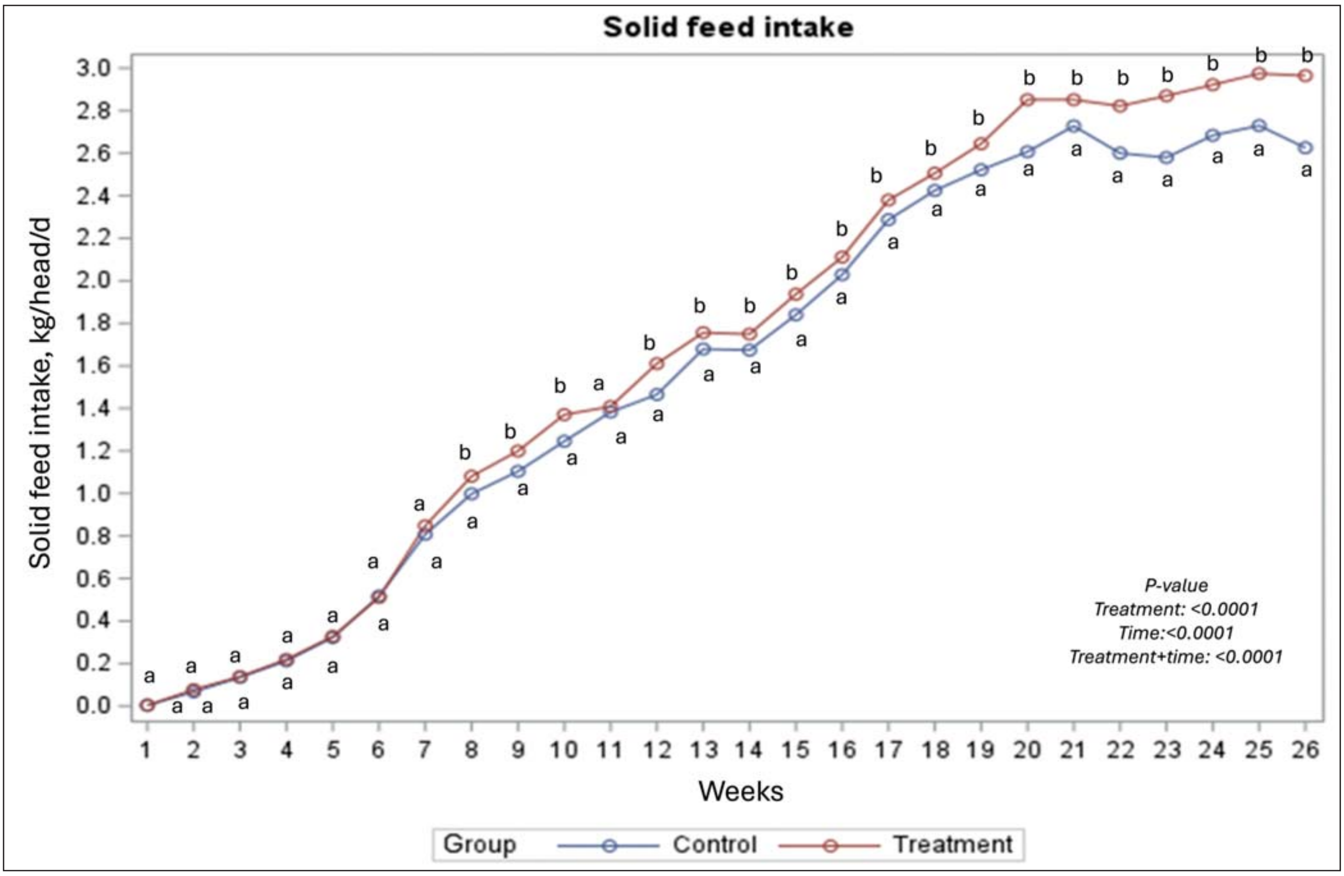

Figure 1 - Trend in solid feed intake along the trial in the group. Different letters (a, b) between rows at the same timepoint highlight a significant different for P<0.05.

nificant differences were detected in terms of incidence of the first episode of bovine respiratory disease (BRD) (85.71 vs 85.82 % in the Control group). However, there was a tendency toward a significant reduction in the incidence of BRD relapses in the Treatment group (13.08 vs 16.4 % on the total animals and 15.17 vs 19.71 % on the first cases of BRD in the Control group) (P=0.0533). No effects were conversely found in terms of incidence of enteric disorders and mortality rate.
The recorded incidence of first cases of BRD can appear to be high, but it is however not uncommon in the white veal calves farming system. In that system, the young age of the animals in union with other critical aspects, such as the commingling of animals from different farms, imprecisions in the administration of colostrum in the farm of origin, transport, and other aspects such as a change in the diet, often lead to a high incidence of health issues, and thus of antimicrobial treatment, especially in the first period of the rearing phase (21, 33). However, the administration of the blend of essential oils, bioflavonoids and tannins tested in the present trial has reduced the incidence of relapses, highlighting a potential positive effect of it on the immune functionality and resilience of the calves, reducing thus the severity of the health impairments.
The bioactive compounds contained in the product, such as bioflavonoids, are reported to have a positive effect on antioxidant status in beef cattle (34). The use of flavonoids and of product that contain flavonoids is also reported to have positive effect on the antioxidant status in pre-weaned and weaned dairy calves (20,35,36).
A high incidence of pathologies is correlated with poorer growth performance, due to both a reduction in feed intake and higher energy devoted to fight the disease and to the immune system. Thus, promoting a better health status both exerting directly antimicrobial and bactericidal properties as well as promoting the antioxidant defences and the immune functionality, can be effective to obtain better production performance, coupled with increased efficiency and reduced needs of antimicrobials. Since the incidence of diseases is correlated to a reduction in the growth efficiency even in veal calves, a potential effect of the treatment on the overall health status, as reported in Table 4, can further explain the better growth performance obtained (21).

Greenhouse gases emissions

Data related to the trend in CH_4 emissions along the rearing period are visible in Figure 2.
As visible in Figure 2, the treatment started to be effective from week 8 of the trial (85.39 *vs* 89.91 g/m^3 in the Control group in week 8) (P<0.0001), leading to a significant effect of the treatment along the entire fattening period. Indeed, considering the entire rearing period, the treatment has led to a significant reduction in the CH_4 emissions (86.20 vs 90.97 g/m^3 in the Control group) (P<0.0001).
This trend can be explained in two ways. Firstly, as previously reported in previous studies done in adult animals, the rumen of an adult animal needs about four weeks to effectively adapt to natural feed additives (37,38). Also, the intake of solid feed, the main driver of CH_4 emissions, is low during the first weeks of the rearing period in veal calves. Their emissions are thus negligible in the first period of the fattening, and even the inclusion of treatments inside the solid feed might have a neg-

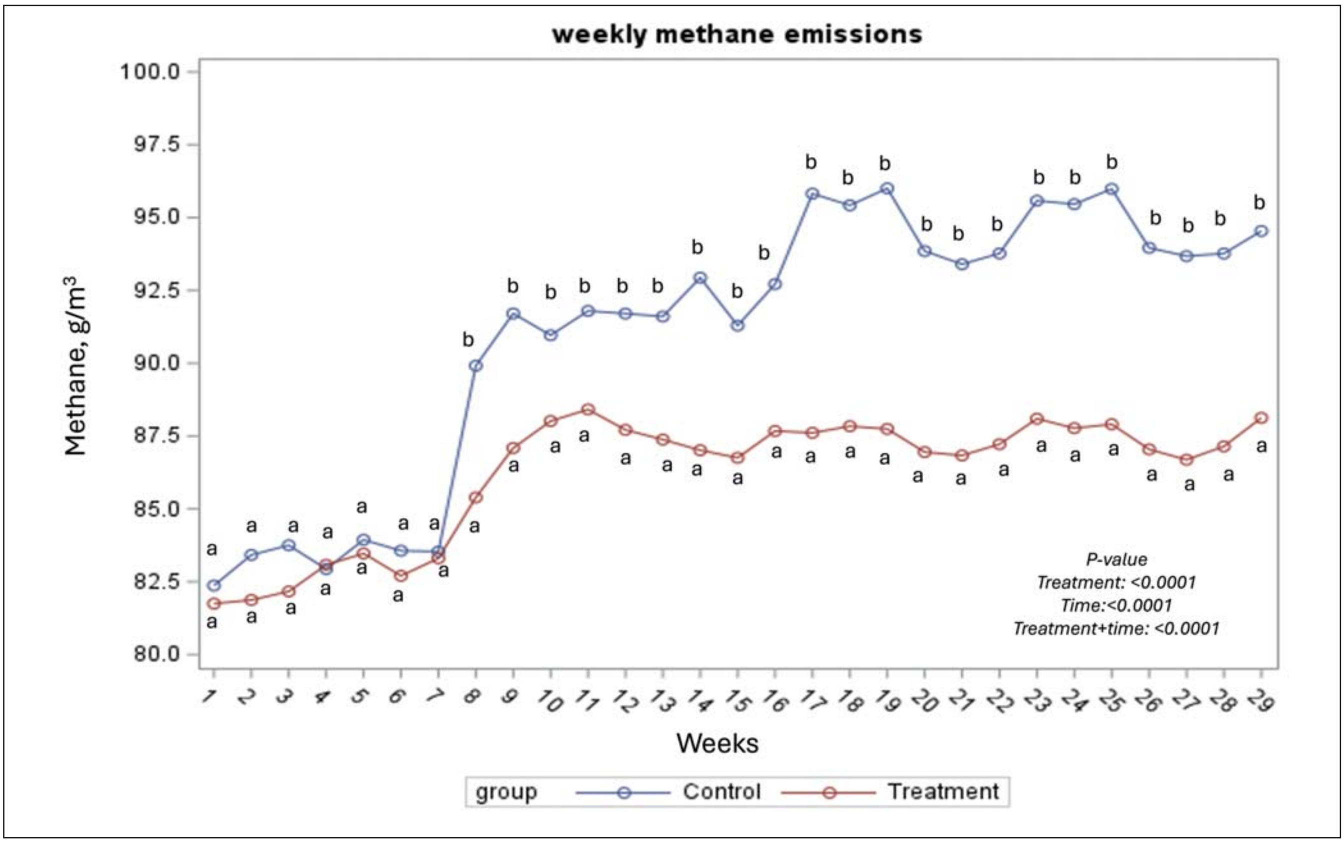

Figure 2 - Trends in methane emissions along the trail in the two groups. Different letters (a, b) between rows at the same timepoint highlight a significant different for P<0.05.

ligible effect during those first phases. Conversely, starting from about the second month of the fattening, the intake of solid feed started to increase, as highlighted also in the present trial, thus becoming an important part of the nutrition of the calves. Starting from that point, the emission of CH_4 might also start to increase, due to the higher quantities assumed, that can lead to a higher production of fermentation byproducts in the rumen (11,12). Moreover, at this point the rumen became more developed and functional, an aspect that might increase the fermentations and thus also the emissions of CH_4 (11,12).
In the present situation, the inclusion of the tested blend of essential oils, bioflavonoids and tannins allowed a reduction of the CH_4 emissions, starting from week 8, moment in which the solid feed intake started to increase. Moreover, treated animals have shown a higher feed intake, and thus the reduction effect on CH_4 emissions caused by the treatment is even amplified. Those results can be mainly ascribed to a positive manipulation of the ruminal microflora, stimulating the development of propionic bacteria. Besides being better from a production perspective, propionate acts also as a hydrogen acceptor in the rumen, subtracting it from the environment. It reduces thus its accumulation in the rumen, that is unfavorable for methanogenic microbes, reducing thus their development (39). Moreover, some essential oils have been reported to have antibacterial properties against methanogenic microbes (40).
Even if the scientific bibliography correlated with the use and role of anti-methanogenic compounds in young calves is still lacking, there are thus evidence that an early administration of some anti-methanogenic compounds in dairy calves can be effective in the manipulation of the ruminal microflora, with also long-lasting effects (5,6). Specifically, the methanogenic bacteria as well as hydrogen-producing ones resulted to be reduced (5,6).

CONCLUSIONS

The results of the present trial highlight that the inclusion of a blend of essential oils, bioflavonoids, and tannins in the solid feed of white veal calves can be an effective strategy to increase the production efficiency, while tackling the use of antimicrobials, improving animal welfare, and counteracting the environmental impacts. Indeed, it has led to better growth performance as a consequence of both improved health and resilience as well as higher gastrointestinal development and functionality. This latter aspect might have led also to a more efficient fermentation of the feeds into valuable nutrients and more effective nutrients, such as propionate and butyrate, reducing thus the production of inefficient byproducts, such as hydrogen, that might increase the production of CH_4.
Also, the economical sustainability can be improved in this sense, with a better growth performance in the Treatment group correlated with a reduction in the need of antibiotics and with a reduction in the incidence of underweight carcasses.

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