

# Effect of Natural Feed Supplement on Methane Mitigation Potential and Performance in Holstein Bull Calves

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**How to cite this paper:** Brand, T., Miller, M. and Kand, D. (2021) Effect of Natural Feed Supplement on Methane Mitigation Potential and Performance in Holstein Bull Calves. *Open Journal of Animal Sciences*, 11, 222-230.

<https://doi.org/10.4236/ojas.2021.112017>

**Received:** January 22, 2021

**Accepted:** April 17, 2021

**Published:** April 20, 2021

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## Abstract

Introduction of solid feeds in the ration of calves has been shown to increase rumen size and stimulate rumen fermentation. With the initiation of bacterial fermentation in the rumen, the intermediary metabolism moves from a glucose-based to a volatile fatty acid-based metabolism, which releases methane. Mootral™ has been identified as a promising plant-derived feed supplement to reduce methane emission in dairy and beef cattle. Therefore, the present study aimed at quantifying and mitigating the methane emissions in calves until the slaughtering age of 28 weeks. The study consisted of 20 Holstein bull calves at a commercial farm, assigned randomly into 2 groups (control n = 10; treatment n = 10), for 2 weeks of adaptation and 8 weeks of sample collection. The calves were fed an increasing amount of milk replacer and *ad libitum* wheat straw. Mootral was fed once a day to the treatment calves. Methane was measured using GreenFeed units where concentrate feed was offered as bait. The calves were weighed at the start and every four weeks during the experiment. The calves in the treatment group had lower methane emissions (54 g/d) compared to the control group (70 g/d), a reduction of 22.8%. In contrast, carbon dioxide emission and dry matter intake did not differ significantly between the study groups. Moreover, no negative impact on the average daily weight gain and carcass weight was observed in Mootral fed calves. Although the methane emission (g/kg body weight) was lower in treatment than in the control group, the absolute difference between the groups narrowed with increasing age of the calves. The results suggest a need to increase the dose in line with the increased body weight and intake of the calves. In conclusion, Mootral effectively reduced methane in calves. Further trials to determine the optimal dose for calves are warranted, and as well studies to investigate if interventions (such as Mootral) applied at an earlier life cycle stage would have an impact on methane emissions at later stages of cat-

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tle's life, would be of scientific interest.

## Keywords

Calves, Methane Emission, Mootral, GreenFeed

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## 1. Introduction

Newly born calves are pre-ruminants and they gradually emerge as ruminants [1]. The transition phase (from the pre-ruminant to the ruminant phase) occurs between 3 and 8 weeks of age, while the ruminant phase begins at about 6 to 8 weeks of age, and a calf usually has a fully developed rumen at 12 weeks of age [2]. Introduction of solid feeds in the ration of veal calves has been shown to increase rumen size and stimulate rumen fermentation [3]. With the initiation, bacterial fermentation in the rumen and the intermediary metabolism moves away from being glucose-based towards being volatile fatty acid-based [4]. This results in volatile fatty acid synthesis by bacterial fermentation in a fully developed rumen, which in due course starts methane (CH<sub>4</sub>) production.

The CH<sub>4</sub> from enteric fermentation in cattle contributes significantly to greenhouse gas emissions, where international conventions aim to reduce these emissions. There are limited strategies for reducing CH<sub>4</sub> from enteric fermentation that can be widely applied. Two such strategies with the greatest potential are diet manipulation and the use of feed supplements. For instance, Moraes *et al.* (2015) investigated the potential of diet manipulation, e.g. in the form of feeding rations with high starch but low fiber content, and concluded that it is possible to reduce enteric CH<sub>4</sub> emissions modestly, yet the cost of implementing this strategy could be up to 49% higher than the current diet formulation [5]. Therefore, feed supplements may be a better alternative to reduce enteric emissions in a cost-effective manner.

Mootral™ (Mootral SA, Rolle, Switzerland), a feed supplement with its main ingredients, garlic granules high in allicin content, and citrus extracts, has shown a potential to reduce CH<sub>4</sub> emissions. Eger *et al.* (2018) showed that Mootral reduced the percentage of methanogens during *in vitro* fermentation, which could be linked to a reduction of enteric CH<sub>4</sub> release and production rate [6]. In addition, Mootral demonstrated a reduction in enteric CH<sub>4</sub> production in lactating cows while increasing milk yield without affecting milk quality in commercial farm conditions [7]. Correspondingly, Mootral has exhibited a reduction in enteric CH<sub>4</sub> yield in beef cattle [8]. Based on these findings, Mootral may also have the ability to impact the CH<sub>4</sub> emission in young animals, such as calves. Studies with feed supplements such as Mootral [7] [8], 3-nitrooxy propanol [9] [10], and essential oils [11] [12] have so far primarily focused on investigating CH<sub>4</sub> emissions, CH<sub>4</sub> intensity per unit of nutrient intake, milk yield or weight gain in adult cattle around the world. However, they have devoted little attention to CH<sub>4</sub>

production in the early ruminating phase of calves (starting at 12 or 13 weeks of age). Thus, the objective of the present short-term study was to understand the effect of Mootral on enteric CH<sub>4</sub> emissions and performance in Holstein bull calves managed at a similar production level in a commercial farm location.

## 2. Materials and Methods

### 2.1. Experimental Design, Animals, and Diet

The present study was conducted between December 2019 and March 2020 over a period of 70 days at a commercial farm in the Netherlands. The study was a randomized block design with 2 weeks of adaptation and 8 weeks of the experimental phase. Twenty Holstein Friesian bull calves of similar age (18 weeks) and weight (mean  $\pm$  standard deviation; 159  $\pm$  9 kg) were selected for this study and were assigned to either the Control group (no feed supplement) or the Mootral group (4 g Mootral per day and head) in a blind test. Both groups of 10 calves were housed in a similar pen and had free access to clean drinking water. Calves were fed the same diet over the entire trial period (**Table 1**). Milk replacer was offered twice a day and was increased with the age of the calves. Wheat straw and concentrate were offered *ad libitum*. Calves in the Mootral group were fed the Mootral feed supplement after the feeding of the milk replacer in the morning by mixing it to feed sugar. Mootral supplement consisted in the major part of dried garlic granules (*Allium sativum*) with an allicin potential of >1%, extracts of bitter oranges (*Citrus aurantium*), and a mixture of saturated fatty acids.

### 2.2. Sample Collection and Analysis

The CH<sub>4</sub> and carbon dioxide (CO<sub>2</sub>) emissions were measured over the whole experimental phase using the GreenFeed system (GF; C-Lock Inc., Rapid City, SD, USA). The GF is an automated system that measures gas fluxes (CH<sub>4</sub>, CO<sub>2</sub>) of individual animals, with a built-in automatic feeder that uses concentrate as bait. Each experimental group had their own GF unit attached to their pen to separate the measurements of both groups and avoid falsified measurements. Calves were equipped with RFID ear tags so that the GF unit could identify each

**Table 1.** Formulation of ration.

Ingredients, in % DM	Control	Mootral
Concentrate <sup>1</sup>	52.34	52.30
Milk replacer <sup>2</sup>	40.68	40.65
Wheat straw <sup>3</sup>	6.98	6.97
Mootral <sup>4</sup>	-	0.08

<sup>1</sup>Contained % dry matter basis: 14% crude protein, 5% crude lipid, 4.8% crude ash, 15.2% neutral detergent fiber; <sup>2</sup>Contained % dry matter basis: 14.4% crude protein, 18% crude lipid, 7.15% crude ash, 0.3% crude fiber; <sup>3</sup>Contained % dry matter basis: 3.2% crude protein, 5.55% crude ash, 63% neutral detergent fiber; <sup>4</sup>Contained % dry matter basis: 16.7% crude protein, 16.6% crude lipid, 2.1% crude ash, 0.6% crude fiber, 0.2% sodium.

individual animal. The farm's usual concentrate was used as bait, and calves were able to visit the GF at free will. The amount of concentrate offered, and the CH<sub>4</sub> and CO<sub>2</sub> emissions at every visit were recorded via internet connection. Only measurements that were longer than 2 minutes were considered for further data analysis.

Body weight (BW) of the calves was measured and recorded once a month. The average daily gain (kg/d) was calculated according to the BW measurements (Final BW minus Start BW divided by days on feed). Solid feed intake of each calf was calculated by adding concentrate, milk replacer and wheat straw intake (kg/d). Calves were slaughtered on the same day at a slaughter facility in the Netherlands at 28 weeks of age. Carcass weight and quality were measured on-site.

### 2.3. Statistical Analysis

All data were analyzed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). The statistical model included treatments as fixed effects with calves and GF unit as random effects. The data of all the studied variables for each calf were averaged across days and were used in the statistical analysis. Means were separated by pairwise t-test (pdiff option of PROC MIXED). Statistical differences were considered significant at  $P \leq 0.05$  and a trend at  $0.05 < P \leq 0.10$ . Data in tables are presented as least squares means.

## 3. Results

The calves' body weight was not significantly different between the dietary treatments both at the start and end of the experiment (**Table 2**). Nevertheless, the body weight showed a tendency for the difference at the start of the experiment ( $P = 0.05$ ), which was not reflected by the end of the experiment ( $P = 0.72$ ). Daily weight gain, carcass weight, carcass conformation, and carcass fat were similar between the dietary treatments ( $P \geq 0.44$ ).

**Table 2.** Effect of Mootral on body weight, body weight gain, carcass gain and slaughter results of Holstein bull calves from 20 to 28 weeks of age.

Variables	Treatment		SEM	P-value
	Control	Mootral		
Start body weight (kg)	163	155	3.70	0.05
Final body weight (kg)	248	245	6.92	0.72
Average daily gain (kg/d)	1.02	1.05	0.06	0.53
Carcass weight (kg)	152	149	4.08	0.44
Carcass gain	0.92	0.93	0.04	0.80
Carcass conformation	11.3	11.4	0.22	0.66
Carcass fat	2.4	2.3	0.22	0.66

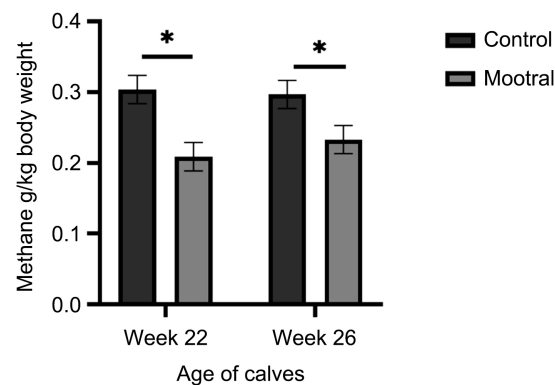
Dry matter intake did not differ between the Control and Mootral calves (**Table 3**). Nevertheless, the CH<sub>4</sub> emission g/d and intensity related to solid intake and body weight were significantly lower by 22.8, 23.8, and 32.3%, respectively, in Mootral compared to Control fed calves ( $P \leq 0.01$ ). In contrast, no difference in CO<sub>2</sub> g/d was observed between the groups.

The CH<sub>4</sub> emission g/kg body weight of the calves was significantly different between Control and Mootral groups at all BW measurement weeks (**Figure 1**). The absolute differences between the dietary treatments in the measurement weeks were greatest in week 22 and lowest in week 26.

**Table 3.** Effect of Mootral on intake, and methane and carbon dioxide emissions from 20 to 28 weeks of age.

Variables	Treatment		SEM	P-value
	Control	Mootral		
<b>Intake</b>				
Solid feed (kg/d)	2.84	2.93	0.14	0.55
Total feed <sup>1</sup> (g/kg <sup>0.75</sup> /d)	77.1	79.3	2.73	0.43
<b>Methane</b>				
g/d	70	54	3.90	<0.01
mg/g solid feed intake	24.8	18.9	1.47	<0.01
g/kg BW	0.31	0.21	0.02	<0.01
g/kg BW gain	69.6	53.3	5.85	0.01
g/kg carcass gain	76.9	60.0	5.85	0.01
<b>Carbon dioxide</b>				
g/d	5081	4841	176.3	0.19
mg/g solid feed intake	1.80	1.68	0.067	0.09
Methane/Carbon dioxide	0.014	0.011	0.0007	<0.01

<sup>1</sup>Total feed intake contains milk replacer, concentrate, and wheat straw.



**Figure 1.** Methane emission g/kg body weight at different ages of the calves fed for control (n = 10) and Mootral (n = 10) based diets.

## 4. Discussion

Dairy and beef production are mainly responsible for the enteric CH<sub>4</sub> emission from livestock [13]. The convention of enteric CH<sub>4</sub> research had focused on the life stages of ruminants when milk is produced, or weight gain is increased. It must be considered that each dairy cow and beef cattle not only emits CH<sub>4</sub> in high-performance phases, but also as soon as the rumen development begins. Studies have confirmed that the same microorganisms present in a mature cattle rumen are already present in very early rumen development stages of calves [14]. Guzman *et al.* (2015) studied the composition of the rumen microbiome of newborn Holstein calves and found that already 20 minutes after birth methanogens were existing [15]. Another experiment with newborn Holstein calves reported that calves were able to produce CH<sub>4</sub> two days after birth [16]. In our study, we showed that veal calves produce and emit a substantial amount of CH<sub>4</sub> (on average 70 g/d and 54 g/d in the Control group and Mootral group respectively) at the age of 20 to 28 weeks. Our findings are in accordance with a study that showed the effect of feeding different amounts of milk replacer to newborn Holstein calves on CH<sub>4</sub> emissions using a respiration chamber [17]. The authors observed that the calves produced CH<sub>4</sub> at the first measurement after 6 weeks which was doubled after 14 weeks. Similarly for other ruminants, the effect of tea saponins and soybean oil supplementation on the CH<sub>4</sub> emissions of lambs also found that young lambs produced substantial amounts of CH<sub>4</sub> (26 L/kg DMI) in early parts of their life [18]. With this proof that young ruminants are capable of producing greenhouse gases such as CH<sub>4</sub>, the need for CH<sub>4</sub> mitigation strategies is even more pressing.

The tested Mootral supplement is a blend of dried garlic granules (*Allium sativum*) with high allicin potential, and citrus extract obtained from bitter oranges (*Citrus aurantium*). Allicin from garlic is known for its antimicrobial and antiviral activity [19], similar effects were observed for polyphenols present in citrus extracts [20]. Although it is difficult to assign the effects of a plant-based blend to its individual ingredients, studies with Mootral suggested, that the combination of the above-mentioned ingredients had CH<sub>4</sub> inhibiting effects in the rumen system. In the present study, the Mootral fed group showed reduced CH<sub>4</sub> emissions of about 23% compared to the Control group. A similar reduction of 23% in CH<sub>4</sub> emissions was observed by Roque *et al.* (2019) who studied the effect of Mootral in a feedlot trial with crossbred steers to examine possible CH<sub>4</sub> reduction, also using the GF system [8]. However, the CH<sub>4</sub> emissions per kg of DMI of the beef steers were lower than those of the calves in the present study, indicating that the production of veal is possibly more emission-intensive than beef production. In lactating dairy cows, Mootral also showed a significant reduction in CH<sub>4</sub> emissions of up to 38% on a commercial farm [7]. Similarly, the effect of Mootral on CH<sub>4</sub> reduction by alteration of the rumen microbiome, particularly the archaeal community, was confirmed by using the *in vitro* RUSITEC technique with rumen fluid from Holstein cows without any negative impact on ru-

men fermentation [6]. Another *in vitro* trial using rumen content of 60 days (*i.e.*, 8.5 weeks) old Holstein calves proved that garlic oil was able to reduce the CH<sub>4</sub> production significantly [21]. As a result, the present and the above-mentioned studies suggest that Mootral supplementation is an effective strategy to reduce CH<sub>4</sub> emissions not only in adults but also in young animals.

Besides reduction in CH<sub>4</sub> emissions, no negative impact of Mootral supplementation on the palatability of the feed and the average daily gain in veal calves was observed in the present study. Similar results for Mootral supplementation were reported on the average daily gain of crossbred steers in an experiment over 12 weeks [8]. Nevertheless, research suggested that garlic had the potential to enhance performance in young cattle. In a study where garlic paste was fed to 18 crossbred calves from the age of 5 days until 2 months of age, the calves had significantly higher daily weight gain when compared to the Control group [22]. However, in the present study, the reduced sample size (10 calves per treatment) may have possibly refrained from detecting such influence on the performance data of the calves.

In the present study, CH<sub>4</sub> emissions (in g/kg BW) from the Control group were always greater than in the Mootral group. However, the emissions differences between both groups decreased in the course of the trial (see **Figure 1**). It may be postulated that as the dosage of the Mootral remained unchanged throughout the experiment (4 g/d), the difference in CH<sub>4</sub> emissions (in g/kg BW) between the studied groups may have decreased. Along the line, other studies have also shown a need for different dosages of CH<sub>4</sub> inhibiting supplements depending on use, body weight, and feed intake of ruminants. For instance, 3-nitrooxypropanol at 150 mg/kg DMI and 200 mg/kg DMI were most effective against CH<sub>4</sub> emissions of dairy cows [10] and beef cattle [23], respectively. Accordingly, it may be indicated that Mootral dosage may need to be selected proportionally to the body weight (growth) of the calves.

## 5. Conclusion

In conclusion, calves fed Mootral have shown a significant reduction in CH<sub>4</sub> emission by 22.8% without being restricted in their performance. Mootral supplementation did not negatively influence the feed intake and carcass characteristics of the veal calves. Smaller differences between the Control and Mootral group on CH<sub>4</sub> emissions in g/kg BW over the course of the trial indicated that a dose adjustment according to the growing BW of the calves could have been necessary. Further research is needed to understand the effect of Mootral in a dose-response manner to further investigate the CH<sub>4</sub> reduction potential in calves.

## Acknowledgements

The authors greatly acknowledge the support of the commercial farm where the study was conducted and Lynette Chew for critically reviewing the manuscript.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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