Impact of inulin and yeast containing synbiotic on calves’ productivity and greenhouse gas production

S. Jonova\(^1\), A. Ilgaza\(^1\), M. Zolovs\(^2\) and A. Balins\(^3\)

1. Faculty of Veterinary Medicine, Preclinical Institute, Latvia University of Life Sciences and Technologies, Jelgava, Latvia; 2. Department of Biosystematics, Institute of Life Sciences and Technology, Daugavpils University, Daugavpils, Latvia; 3. Research Laboratory of Biotechnology, Division of Molecular Biology and Microbiology, Latvia University of Life Sciences and Technologies, Jelgava, Latvia.

Corresponding author: M. Zolovs, e-mail: maksims.zolovs@du.lv

Co-authors: SJ: sintija.jonova@llu.lv, AI: aija.igaza@llu.lv, AB: andris.balins@llu.lv

Received: 20-03-2020, Accepted: 21-04-2020, Published online: 02-06-2020
doi: www.doi.org/10.14202/vetworld.2020.1017-1024 How to cite this article: Jonova S, Ilgaza A, Zolovs M, Balins A (2020) Impact of inulin and yeast containing synbiotic on calves’ productivity and greenhouse gas production, Veterinary World, 13(6): 1017-1024.

Abstract

Aim: The research aimed to determine the impact of synbiotic: 6 g of prebiotic inulin and 5 g of probiotic Saccharomyces cerevisiae strain 1026 on calves’ productivity and greenhouse gas (GHG) production.

Materials and Methods: The research was conducted with 10 Holstein Friesian and Red Holstein (Bos taurus L.) crossbreed calves of mean age 33±6 days and initial body weight 73.4±12.75 kg. We added the synbiotic into the diet of five dairy crossbred calves (SynG) and five calves in control group (CoG) received non-supplemented diet. The duration of the experiment was 56 days. The weight of calves and amount of methane (CH\(_4\)) and carbon dioxide (CO\(_2\)) in the rumen were determined on day 1, 28, and 56. On day 56, three calves from each group were slaughtered. Meat samples were assessed for some indicators of meat quality. The main methanogens were detected in the rumen fluid and feces.

Results: The weight gain during the whole experiment period of 56 days was higher in the SynG (62.6±13.75 kg) compared to CoG (36.8±7.98 kg) calves (p<0.01). There were no significant differences in the levels of protein (%), fat (unsaturated and saturated – %), and cholesterol (mg/100 g) in meat samples from both groups. At the end of the experiment, the amount of CH\(_4\) in calves’ rumen in CoG was higher (Me=792.06 mg/m\(^3\), IQR 755.06-873.59) compared to SynG (Me=675.41 mg/m\(^3\), IQR 653.46-700.50) group (p<0.01). The values for CO\(_2\) were also increased in CoG (Me=4251.28 mg/m\(^3\), IQR 4045.58-4426.25) compared to SynG (Me=3266.06 mg/m\(^3\), IQR 1358.98-4584.91) group (p=0.001). There were no significant differences in the calves’ weight and certain methanogen species in rumen liquid and feces on the 56th day of the experiment. Significantly higher results in the parameter total prokaryotes (V3) (bacteria+archaea) in rumen fluid were in SynG, whereas significantly higher results in the parameter total methanogens Met630/803 in rumen fluid were in CoG, p<0.05.

Conclusion: The main results showed that the synbiotic can increase the daily weight gain in calves and decrease the amount of GHG in rumen but does not impact different methanogen species in rumen liquid and feces and meat protein, fat, and cholesterol levels.

Keywords: calves, greenhouse gases, inulin, productivity, Saccharomyces cerevisiae, symbiotic.

Introduction

The United Nations has calculated that the global demand for food will double by the year 2050 when the population is going to increase up to 9.8 billion. This demand is going to be a great challenge for agricultural industries as the world will need extra food for the growing population. The provided food will have to be healthy, nutritious, and sustainably produced [1]. The greatest challenge will be to reach this goal and at the same time reduce the emission of greenhouse gases (GHGs) from the agricultural sector. The global temperature of our planet has increased by 0.85° since 1880 mainly due to human activity [2]. Livestock products are responsible for increased GHG emissions compared to other food sources. The emissions of dairy cattle are the result of complex biological processes that occur in animal digestive system. The most important is methane (CH\(_4\)), which is produced as a by-product of the digestion processes [1]. The second GHG emitted from dairy cattle that contributes to global warming is carbon dioxide (CO\(_2\)). The global warming potential of CH\(_4\) is 21 times more than CO\(_2\) [3]. In general, the CO\(_2\) produced through respiration processes is not considered as a great source of GHG emissions, since it is assumed that the observed amount of CO\(_2\) by plants is equivalent to the amount emitted by livestock. Furthermore, the consumed carbon is used in animal tissues and products, such as milk [4].

Nowadays, researchers are working on ways of reducing GHG emissions from all agricultural...
sectors, including livestock farming and increasing animal productivity. The aim is to produce less CH₄ per unit of meat or milk [5]. Rumen methanogens use H₂ and CO₂ produced by other fermentative members of the ruminal microbiome, to create CH₄ [6]. This gas not only negatively impacts our surrounding environment but also causes energy loss to animals. It is proven that about 2-12% of the ingested feed energy is lost as CH₄ [7]. Changes in animal diet and addition of different feed additives have been identified as main ways for the mitigation of CH₄ production and the improvement of animal health and productivity [1]. The prebiotics or oligosaccharides are non-digestible carbohydrates commonly used in the non-ruminants for the improvement of gut health and feed utilization. They are also used in rumen manipulation along with nitrates, probiotics, and yeast since they have the potential to reduce CH₄ production [5]. At present, animal researchers are exploring the efficiency of prebiotic inulin for modulating the gut ecosystem of both ruminants and non-ruminants. In ruminants, the prebiotic reduces rumen ammonia nitrogen, CH₄ production, increase microbial protein synthesis, and live weight gains in calves [8-10]. On the other hand, probiotics have been defined as living microorganisms that when contained in the feed of animals positively affect the host by improving its digestive system and its weight gain [11,12]. One of the promising probiotics that could improve the health and performance of young calves is the live yeast Saccharomyces cerevisiae. Dietary supplementation with S. cerevisiae might increase feed intake and energy utilization, strengthen the immune response, and reduce the incidence in diseases of young calves [13]. One potential mode of the action of S. cerevisiae is to scavenge oxygen within the rumen creating a more anaerobic environment, which is required by ruminal microorganisms [14]. S. cerevisiae is also considered to provide nutrients, such as organic acids, B vitamins, and amino acids, that stimulate microbial growth in the rumen, thereby indirectly stabilizing ruminal pH [15]. Yeast also has the potential to alter the fermentation process in the rumen in a manner that reduces the formation of CH₄ gas. It has been reported a shift in H₂ utilization from methanogenesis to reductive acetogenesis by yeast in in vitro experiments [6].

As previously described, prebiotic inulin and probiotic S. cerevisiae when used alone positively impact animal growth rate and reduce the production of CH₄, but no research has been conducted so far using these two feed additives together as a synbiotic. This research aimed to measure the amount of CH₄ and CO₂ in the rumen and feces after synbiotic addition that contained prebiotic inulin and probiotic S. cerevisiae. At the same time, the productivity of calves by comparing the live weight gain and several parameters of meat quality was evaluated.

Materials and Methods

Ethical approval

All procedures performed in the present study were in accordance with the ethical standards. Research Committee of the Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies approved this study (protocol no. 2017/2).

The method of the collection of rumen fluid was invasive (puncturing the abdomen) and this caused some pain in calves; moreover, some calves were slaughtered at the end of the experiment to obtain samples of meat and gastrointestinal tract for histological examination. Following the ethical requirements to minimize the number of animals used in experiments, we chose to organize as small groups as possible (five animals per group).

Study period and location

The research was conducted in a dairy farm in Latvia, Saldus District. The research was performed from March until the end of April 2018.

Animals

Ten clinically healthy randomly selected Holstein Friesian and Red Holstein (Bos taurus L.) crossbred calves with a mean age of 33±6 days and initial body weight of 73.4±12.75 kg were used in the present study. All calves were housed in groups in a partly closed pen in a farm. After birth, all calves received colostrum, and for the next 5 days, calves received whole milk (3.5 L twice a day) and later the milk replacer in a dosage appropriate to their age and weight. Within the age of 4-8 weeks, calves received 8 L of milk replacer per day and a pre-starter diet without restriction (around 0.5 kg/calf/day). After the age of 8 weeks, calves received approximately 1.5 kg of barley flour and 6 L of milk replacer per day. During the experiment, calves had free access to hay and water.

Experimental design

Calves were allocated into two groups: Five calves in the control group (CoG) receiving a standard diet and five calves that additionally received a synbiotic which consisted of two different products; 12 g of flour of Jerusalem artichoke (Helianthus tuberosus L.) per head containing 6 g of prebiotic inulin (produced in Latvia, at the University of Latvia, Institute of Microbiology and Biotechnology) and probiotic 5 g of a yeast culture based on S. cerevisiae strain 1026 (Yea-Sacc®, Alltech Inc., USA) (SynG). The prebiotic and probiotic were added to barley flour once a day in the morning.

The duration of the experiment was 8 weeks (56 days). We measured the amount of CH₄ and CO₂ in the rumen and determined the weight of calves. The full technique is described in our previous study [16]. The samples from calves’ rumen were evaluated 3 times during the research with an interval of 4 weeks – at the 1st, 28th, and 56th days of the research.

At the end of the experiment, three calves from each group were slaughtered at certified slaughterhouse
following all guidelines of humane slaughter. Meat samples from the longissimus muscle were collected and sent to the accredited laboratory for the assessment of some indicators of meat quality (amount of protein [LVS ISO 937:1978] [Kjeldahl method], fat [LVS ISO 1443:1973] [Soxhlet method], [Gravimetry], fatty acids [BIOR-T-012-131-2011] [Gas chromatography], and cholesterol [BIOR-T-012-132-2011] [Gas chromatography]).

Mixed stool samples of each calf group and individual rumen samples of all calves’ group were used to detect methanogens. DNA was isolated by QIAamp® DNA Stool Mini Kit. There was used 200 mg of frozen samples and processed according to the manufacturer’s instructions. DNA amount and purity were verified by NanoDrop-1000, Thermo Fisher Scientific Inc. spectrophotometer. Isolated DNA samples were stored at −20°C till future analyzes.

Specific primer sets were used to detect methanogens. The primer sequences for the methanogens were as follows:

**Total methanogens (rrs):**
- Met630F: 5'GGATTAGATACCCCGGTGTAG-3'; Met803R: 5'GGTTGARTCCATTAACCGCA-3'.
- Total prokaryotes (rrs, reference gene):
  - V3-F: 5'CCTACGGGAGGCAGCAG-3'; V3-R: 5'ATTACCGCGGTGTCTGG-3'.
- Methanosphaera stadtmanae (rrs):
  - Stad-F: 5'CTTAATATAAGAATTTGAAGTTG-3'; Stad-R: 5'TTGCATTACTACCGTGCAAGAT-3'.
- Methanobrevibacter ruminantium (rrs):
  - Rum16S: 5'TTCGTTACTCACCGTCAAGAT-3'.
- Methanobrevibacter smithii (rrs):
  - Smit.16S-740F: 5'CCTACGGGAGGCAGCAG-3'; Smit.16S-862R: 5'CTTAACTATAAGAATTGACTGG-3'; Smit.16S-FAM: 5'CCGTCAGGTTCGTTCCAGTTAG-3'.

**Total prokaryotes (rrs, reference gene):**
- V3-F: 5'CCTACGGGAGGCAGCAG-3'; V3-R: 5'ATTACCGCGGTGTCTGG-3'.
- Methanosphaera stadtmanae (rrs):
  - Stad-F: 5'CTTAATATAAGAATTTGAAGTTG-3'; Stad-R: 5'TTGCATTACTACCGTGCAAGAT-3'.
- Methanobrevibacter ruminantium (rrs):
  - Rum16S: 5'TTCGTTACTCACCGTCAAGAT-3'.
- Methanobrevibacter smithii (rrs):
  - Smit.16S-740F: 5'CCTACGGGAGGCAGCAG-3'; Smit.16S-862R: 5'CTTAACTATAAGAATTGACTGG-3'; Smit.16S-FAM: 5'CCGTCAGGTTCGTTCCAGTTAG-3'.

**Polymerase chain reaction (PCR) was performed using QuantiNova™ Probe PCR Kit and QuantiNova® SYBR® Green PCR Kit following manufacturer’s instructions.**

**Amplification of DNA was performed in a Rotor-Gene Q real-time PCR cycler using the following conditions:**
- Initial denaturation at 95°C for 2 min followed by 40 cycles of denaturation (95°C for 5 s) and annealing (60°C for 10 s [total methanogens [rrs], total prokaryotes, and *M. stadtmanae*] and 60°C for 5 s [*M. ruminantium* and *M. smithii*]).
- Methanogen levels were estimated as the value of Ct (PCR cycle number at which sample’s reaction curve intersects the threshold line) was <25 – strong positive, <30 positive, <35 weak positive, and >35 very weak positive.

**Statistical analysis**

The assumption of normal data distribution was assessed by Shapiro–Wilks’s test and visual inspection of their histograms and normal Q-Q plots. The assumption of homogeneity of variances was tested by Levene’s test. To determine whether there were any statistically significant differences between three independent groups, we used Kruskal–Wallis H test with pairwise comparisons using Dunn’s procedure [17] with a Bonferroni adjustment. To determine whether there are any statistically significant differences between the two groups, we used the Mann–Whitney U-test or the independent samples t-test. The measure of the strength and direction of the association between two continuous or ordinal variables was evaluated by the Spearman’s rank-order correlation. Those tests were carried out using SPSS Statistics version 22 (IBM Corporation, Chicago, Illinois). All statistical analyses were performed at P=0.05 and data are presented as means ± standard deviation (SD).

**Results**

Weight gain values between 1st-28th, 28th-56th, and 1st-56th days are presented in Table-1. The data of initial and daily live weight gain were normally distributed, and there was the homogeneity of variances. There were no statistically significant differences in mean live weight gain and mean daily weight gain between groups during the 1st month (period 1st-28th day) (p>0.05). However, independent samples t-test showed that the weight gain during the whole research was higher in the SynG (62.6±13.7 kg) than CoG (36.8±7.98 kg) calves (p<0.01). The mean daily weight gain was also greater in SynG (0.7±0.14 kg) than CoG (0.7±0.14 kg) calves (p<0.01).

**Table-1: Effect of synbiotic supplementation on growth performance parameters of calves.**

| Parameters | CoG | SynG | p-value |
|------------|-----|------|---------|
| Initial mean live weight (kg±SD) | 79.4±10.52 | 67.4±12.83 | 0.145 |
| Mean live weight gain (kg±SD) | 17.6±4.92 | 21.8±4.81 | 0.210 |
| 1st-28th research days | 19.2±4.96 | 40.8±11.16 | 0.004* |
| 28th-56th research days | 36.8±7.98 | 62.6±13.75 | 0.007* |
| 1st-56th research days | 116.2±16.30 | 130.0±25.22 | 0.334 |
| Final mean live weight (kg±SD) | 0.6±0.17 | 0.8±0.17 | 0.216 |
| Mean daily weight gain (kg/day) | 0.7±0.17 | 1.5±0.39 | 0.004* |
| 1st-28th research days | 0.7±0.14 | 1.1±0.24 | 0.007* |

*Significant at p<0.05. CoG=Control group, SynG=Synbiotic group, SD=Standard deviation*
Meat quality was evaluated as the levels of protein (%) and fat (unsaturated and saturated – %) and cholesterol (mg/100 g). There were no statistically significant differences between all parameters in meat samples obtained from calves from groups CoG and SynG (Table-2).

The data of CO\textsubscript{2} and CH\textsubscript{4} concentration in calves’ rumen during the 1\textsuperscript{st}, 28\textsuperscript{th}, and 56\textsuperscript{th} days are presented in Table-3. There were statistically significant differences in the mean amount of CH\textsubscript{4} on days 28 and 56 of the experiment between the two groups. The higher amounts of CH\textsubscript{4} were observed in CoG group (p<0.001). The levels of CO\textsubscript{2} were also significantly higher in CoG in all sampling days.

The mean CH\textsubscript{4} production in calves’ rumen per kg of body weight in CoG at the end of the experiment was also significantly higher in control than synbiotic group (Me=7.10 mg/m\textsuperscript{3}, interquartile range [IQR] 5.61-9.44 vs. Me=5.46 mg/m\textsuperscript{3}, IQR 4.60-5.95, respectively, p<0.05). On the other hand, the mean CO\textsubscript{2} level in calves’ rumen per kg of body weight at the end of the experiment was not significantly different between the two groups (Table-4).

Table-2: Effect of synbiotic supplementation on meat quality traits of calves.

| Parameter | CoG | SynG | p-value |
|-----------|-----|------|---------|
| Protein (%) | 20.1±1.05 | 20.6±0.80 | 1.000 |
| Fat (%) | 1.0±0.03 | 1.8±0.85 | 0.700 |
| Unsaturated | 57.5±1.75 | 52.7±2.73 | 0.100 |
| Saturated | 42.5±1.75 | 48.1±2.76 | 0.100 |
| Cholesterol (mg/100 g) | 60.1±1.10 | 56.6±0.15 | 0.100 |

*Significant at p<0.05. CoG=Control group, SynG=Synbiotic group

No correlation was found between the level of CH\textsubscript{4}, CO\textsubscript{2} gases, and animal weight on day 1, 28, and 56.

There were no significant differences in the calves’ weight and certain methanogen species in rumen liquid and feces on the 56\textsuperscript{th} day of the experiment. Significantly higher results in the parameter total prokaryotes (V3) (bacteria+archaea) in rumen fluid were in group SynG, whereas significantly higher results in the parameter total methanogens Met630/803 in rumen fluid were in group CoG, p<0.05 (Table-5).

Discussion

Different feed additives such as prebiotics, probiotics, and their combination synbiotics have been used in farm animals to improve their growth performance. Based on our results, we can propose that synbiotic (prebiotic inulin + probiotic S. cerevisiae strain 1026) significantly increases live weight gain in calves since increased daily weight gain was observed in SynG calves from the 1\textsuperscript{st} to 56\textsuperscript{th} day of the experiment.

Many studies have proved that different prebiotics and probiotics individually and in various combinations can positively affect the growth rate of different animals. For example, Miguel et al. [18] found that inclusion of the prebiotic mannann oligosaccharide (MOS) at different inclusion levels on an as-fed basis (0.1-0.4%) into piglet diet for 14-56 days can improve their growth rate. Similar conclusions were reached by Tang et al. [19] that fed piglets for 14 days with dietary supplements of oligosaccharides chitosan 0.025% and galacto-mannan-oligosaccharides 0.20%, and the experimental group showed an improved growth rate.

Table-3: Effect of synbiotic supplementation on the mean amount of CH\textsubscript{4} (mg/m\textsuperscript{3}) and CO\textsubscript{2} (mg/m\textsuperscript{3}) in calves’ rumen.

| Parameters (mg/m\textsuperscript{3}) | Day of experiment | CoG | SynG | p-value |
|--------------------------------------|-------------------|-----|------|---------|
| CH\textsubscript{4} | 1\textsuperscript{st} | 811.50 | 107.87-870.45 | 790.18 | 442.75-1032.87 | 0.059 |
| | 28\textsuperscript{th} | 1052.94 | 983.33-1111.89 | 659.11 | 565.04-1015.32 | <0.001* |
| | 56\textsuperscript{th} | 792.06 | 755.06-873.59 | 675.41 | 653.46-700.50 | <0.001* |
| CO\textsubscript{2} | 1\textsuperscript{st} | 3258.54 | 2864.08-3506.88 | 2701.65 | 2419.45-3042.81 | <0.001* |
| | 28\textsuperscript{th} | 4618.15 | 4378.59-4756.74 | 4263.82 | 3553.29-4584.91 | <0.001* |
| | 56\textsuperscript{th} | 4251.28 | 4045.58-4426.25 | 3266.07 | 1358.98-4584.91 | <0.001* |

*Significant at p<0.05. CH\textsubscript{4}=Methane, CO\textsubscript{2}=Carbon dioxide, CoG=Control group, SynG=Synbiotic group, Q1-Q3=Quartile 1-Quartile 3

Table-4: Effect of synbiotic supplementation on the mean amount of CH\textsubscript{4} (mg/m\textsuperscript{3}) and CO\textsubscript{2} (mg/m\textsuperscript{3}) in calves’ rumen on 1 kg body weight.

| Parameters | Day of experiment | CoG | SynG | p-value |
|------------|-------------------|-----|------|---------|
| CH\textsubscript{4}kg | 1\textsuperscript{st} | 9.63 | 1.17-9.86 | 12.95 | 3.94-16.75 | 0.421 |
| | 28\textsuperscript{th} | 10.33 | 9.55-10.90 | 7.58 | 5.95-10.88 | 0.222 |
| | 56\textsuperscript{th} | 7.10 | 6.35-9.19 | 5.46 | 4.60-5.95 | 0.032 |
| CO\textsubscript{2}kg | 1\textsuperscript{st} | 38.36 | 36.32-39.26 | 44.28 | 29.47-46.0 | 0.548 |
| | 28\textsuperscript{th} | 46.42 | 42.58-48.61 | 53.96 | 37.59-54.71 | 0.841 |
| | 56\textsuperscript{th} | 36.72 | 34.63-37.07 | 38.47 | 9.61-62.95 | 0.917 |

*Significant at p<0.05. CH\textsubscript{4}=Methane, CO\textsubscript{2}=Carbon dioxide, CoG=Control group, SynG=Synbiotic group, Q1-Q3=Quartile 1-Quartile 3
Several studies show the positive effect of prebiotic inulin on calves’ growth performance. For example, 36 Holstein Friesian breed calves that received inulin from the 1st to the 56th day of life at the dosage 6 g/day/head showed higher final body weight than animals that did not receive inulin [20].

Our results coincide with that of Lesmeister et al. [21] and Roodposhti and Dabiri [22]. Lesmeister et al. [21] noted an average daily weight gain improvement by 15.6% for 2% yeast (S. cerevisiae) treatment in 42 days long experiment, and Roodposhti and Dabiri [22] concluded that the adding of probiotic at 1 g (Protexin®; multi-strain probiotic contains 7 bacteria strains and 2 yeast strains with 2×10⁹ cfu/mL) and prebiotic at 4 g (a commercial product which contains polysaccharides of S. cerevisiae cell wall) per day for 8 weeks (symbiotic) to calves’ feed can significantly improve their average daily weight gain.

Many studies focus on the impact of prebiotics and probiotics on meat quality of different animal species. Most of them showed that prebiotics and probiotics do not affect meat quality. For example, addition of a probiotic Lactobacillus reuteri (2.5×10⁸ cfu/mL) to broiler chicken diet over the 42 days of the experiment did not affect meat quality [23]. Another study with inclusion of probiotic S. cerevisiae 1 g/kg of diet and probiotic MOS 1 g per kg of diet into turkey diet for 10 weeks also showed that these feed additives do not influence the different parameters of meat quality, including the amount of protein [24].

Raghebian et al. [25] found that probiotic S. cerevisiae 3 g and 4.5 g/lamb/day in 84 days long experiment did not significantly impact the amount of fat in lamb meat. Similar results were presented by Gadekar et al. [26] who added probiotic Lactobacillus acidophilus culture (3.6×10⁸ cells/mL) to lamb diet at dosages 1.0, 1.5, and 2.0 ml/kg body weight for 167 days. Tufarelli et al. [27] in a 12-week long study with pigs that were supplemented with a probiotic blend (Streptococcus thermophilus DSM 32245, a mixture of two strains Bifidobacterium animalis ssp. lactis DSM 32246 and DSM 32247, L. acidophilus DSM 32241, Lactobacillus helveticus DSM 32242, Lactobacillus paracasei DSM 32243, Lactobacillus plantarum DSM 32244, and Lactobacillus brevis DSM 27961) at the dosage 100 mg/kg of body weight observed that the crude protein content increased significantly in pigs fed a probiotic blend; however, no significant differences were observed on meat crude fat content. In our study, we did not record any statistically significant differences in such meat quality parameters as amount of protein (%) and fat (unsaturated and saturated – %) and cholesterol (mg/100 g).

It has been described that probiotic S. cerevisiae can use oxygen within the rumen and by this way, a more anaerobic environment is created, which is required by ruminal microorganisms [14]. Yeast also has the potential to change the fermentation process in the rumen and possibly stimulate the aceticogenic bacteria to compete with methanogens or to cometabolize H₂, thereby reducing the formation of methane gas [6,28,29].

Lynch and Martin [28] in their in vitro experiment found a reduction in CH₄ gas production by 20% after 48 h of incubation of mixed rumen microorganisms containing alfalfa and a live yeast product at concentration of 0.35 and 0.73 g/L. Frumholtz et al. [30] also reported outstanding results in another in vitro experiment. Authors used the probiotic Aspergillus oryzae, and CH₄ production decreased in the experimental group due to the reduction of the protozoal population (45%). These findings are consistent with those of Hernández et al. [31] that have used rumen inoculum of 60 day-old calves supplemented with S. cerevisiae (0, 2, and 4 mg/g of dry matter) and incubated it for 70 h. These results support our findings; a significant reduction of the amount of CH₄ and CO₂ in calves which received symbiotic consisting of prebiotic inulin and probiotic S. cerevisiae was observed.

However, our findings are inconsistent with that of Takahashi et al. [32]. They conducted a 4×4 Latin square design experiment with sheep (each test period consisted of 9 days with 7 days for adjustment to feeds) and used a mixture of Bacillus subtilis, Bacillus cereus, Bacillus thuringiensis, Pseudomonas fluorescens, Streptomyces cellulosae, Streptomyces albidoflavus, and Saccharomyces lipolytica at the dosage 87 mg/kg body weight and observed an increase in the production of CH₄ by 18%.

In our previous 56 days long research in which we have used the flour of Jerusalem artichoke at the doses...
of 12 g (inulin content 6 g) and 24 g (inulin content 12 g), no significant reduction in CH\(_4\) and CO\(_2\) gases in calves’ rumen was recorded [16,33]. These results suggest that sole supplementation with the probiotic inulin does not affect the production of CH\(_4\) and CO\(_2\).

Rumen has a high microbial population density comprised of different prokaryotes, eukaryotes, methanogenic archaea, and bacteriophages [34]. The ruminant species have different methanogen populations, for example, M. ruminantium and Methanobrevibacter mobile are major methanogens in the ovine rumen [35]. In the cow rumen, Methanobrevibacter seems to be the dominant genus of the archaeal domain [36,37]. In an experiment with feedlot cattle, Wright et al. [38] identified following major methanogens in the rumen: M. ruminantium, Methanobrevibacter thaueri, M. smithii, and M. stadtmanae. These findings are consistent with those of Whitford et al. [39]; however, M. ruminantium were the most abundant rumen methanogen followed by M. stadtmanae in that study.

Methanogens produce methane under highly anaerobic conditions [34]. For example, M. smithii produce CH\(_4\) from CO\(_2\), H\(_2\), and formate, but M. stadtmanae produce methane only through reduction of methanol with H\(_2\) [40]. In cows’ rumen, certain groups of Methanobrevibacter species (M. smithii, Methanobrevibacter gottschalkii, Methanobrevibacter millerae, and M. thaueri) are associated with high production of CH\(_4\) [41-43].

S. cerevisiae affects gut microbiota and morphological development in young calves [44]; however, the results of studies on the impact of probiotic S. cerevisiae on ruminal CH\(_4\) production are controversial. Five days long in vitro study revealed that S. cerevisiae increases the growth of acetogenic bacteria that compete methanogens by utilizing H\(_2\) and CO\(_2\) to produce acetate [6]. Ogunaide et al. [45] stated that yeast S. cerevisiae at the dosage 15 g/day might increase the amount of ruminal CH\(_4\) produced in steers in a 25-day long experiment due to the increased abundance of M. ruminantium. Ding et al. [46] reported that yeast S. cerevisiae could increase rumen bacteria, fungi, and protozoa in steers receiving yeast supplementation (8×10\(^6\) CFU/h/day through the ruminal fistula) following a two-period crossover design (four phases, each lasted 17 days); also, we recorded that S. cerevisiae could increase the number of total prokaryotes (bacteria and archaea). Galindo et al. [47] documented the reduction of methanogens and ruminal methanogenesis in 24 h long in vitro experiment by adding S. cerevisiae on star grass (Cynodon nemfuenis L.) which was used as a substrate to be fermented, a finding that is in contrary to the results of our study. We recorded a significantly higher amount of total methanogens in calves of the CoG. However, separate methanogen species, which are considered to be the primary CH\(_4\) producers in the rumen (based on information provided before), were in higher amount in symbiotic group. We can assume that the increase of total methanogens in calves from the CoG is due to other species not examined in our study.

**Conclusion**

We conclude that symbiotic containing 6 g of prebiotic inulin and 5 g of probiotic S. cerevisiae strain 1026, significantly increase the mean daily weight gain in calves. This symbiotic impacts the amount of CH\(_4\) and CO\(_2\) gases by substantially decreasing their level in the rumen of calves; however, no correlation was found between these gases and animal weight. Furthermore, the symbiotic does not impact different methanogen species in rumen liquid and feces, and these methanogens do not have any correlation with calves’ weight or amount of produced methane in the rumen. Inulin and yeast S. cerevisiae do not have any impact on meat quality parameters, such as protein (%), fat (unsaturated and saturated – %), and cholesterol (mg/100 g) levels. The results of this study showed a significant increase in live weight gain and reduction of GHG emissions in calves; therefore, further research is warranted to elucidate the mechanisms of symbiotic activity.

**Authors’ Contributions**

SJ collected the samples, performed the clinical examination of calves, performed the analysis of rumen gases, and drafted the paper and revised it. AI designed the concept for this research and scientific paper. MZ performed the statistical analysis of all data. AB performed the analysis of methanogens. All authors read and approved the final manuscript.

**Acknowledgments**

This research has been supported by the National Research Program, Agricultural Resources for Sustainable Production of Qualitative and Healthy Foods in Latvia (AgroBioRes), Project No. 3 LIVESTOCK (VPP-20142018).

**Competing Interests**

The authors declare that they have no competing interests.

**Publisher’s Note**

Veterinary World remains neutral with regard to jurisdictional claims in published institutional affiliation.

**References**

1. Gerber, P.J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkmans, J., Falucci, A. and Tempio, G. (2013) Tackling Climate Change Through Livestock-a Global Assessment of Emissions and Mitigation Opportunities. Food and Agriculture Organization of the United Nations (FAO), Rome.

2. Field, C.B., Barros, V.R., Dokken, D.J., Mach, K.J., Mastrandrea, M.D., Bilir, T.E., Chatterjee, M., Ebi, K.L., Estrada, Y.O., Genova, R.C., Girma, B., Kissel, E.S., Levy, A.N., MacCracken, S., Mastrandrea, P.R. and White, L.L. (2014) IPCC (intergovernmental panel on climate change), climate change 2014: Impacts, adaptation,
and vulnerability. Part A: Global and sectoral aspects. In: Contribution of Working Group II to the 5th Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom, New York, USA. p1132.

3. Moumen, A., Azizi, G., Chekrouk, B. and Baghmour, M. (2016) The effects of livestock methane emission on global warming: A review. Int. J. Glob. Warming, 9(2): 229-253.

4. UNFCCC (1998) Kyoto Protocol to the United Nations Framework Convention on Climate Change Adopted at COP3 in Kyoto. UNFCCC, Japan.

5. Kataria, R. (2015) Use of feed additives for reducing greenhouse gas emissions from dairy farms. Microbiol. Res., 6(1): 19-25.

6. Chaucheayas, F., Fonty, G., Bertin, G. and Gouet, P. (1995) In vitro H$_2$ utilization by a ruminal acetogenic bacterium cultivated alone or in association with an archaea methanogen is stimulated by a probiotic strain of Saccharomyces cerevisiae. Appl. Environ. Microbiol., 61(9): 3466-3467.

7. Johnson, K.A. and Johnson, D.E. (1995) Methane emissions from cattle. J. Anim. Sci., 73(8): 2483-2492.

8. Aré, A. and Ilgaza, A. (2016) Different Dose Inulin Feeding Effect on Calf Digestion Canal State and Development. Proceedings of the 22nd International Scientific Conference Research for Rural Development, Jelgava, Latvia. p116-118.

9. Jonova, S., Ilgaza, A. and Grinfelde, I. (2017) Methane Mitigation Possibilities and Weight Gain in Calves Fed with Prebiotic Inulin. Proceedings of the 23rd International Scientific Conference Research for Rural Development, Jelgava, Latvia. 265-270.

10. Samanta, A.K., Jayapal, N., Senani, S., Kolte, A.P. and Sridhar, M. (2013) Prebiotic inulin: Useful dietary adjuncts to manipulate the livestock gut microflora. Braz. J. Microbiol., 44(1): 1-14.

11. Salminen, S., Ouwehand, A., Benno, Y. and Lee, Y.K. (1999) Probiotics: How should they be defined? Trends Food Sci. Tech., 10(3): 107-110.

12. Simon, O., Jadams, A. and Vahjen, W. (2001) Probiotic feed additives-effectiveness and expected modes of action. J. Anim. Feed Sci., 10(1): 51-67.

13. Magalhães, V.J.A., Susca, F., Lima, F.S., Branco, A.F., Yoon, I. and Santos, J.E.P. (2008) Effect of feeding yeast culture on performance, health, and immunocompetence of dairy calves. J. Dairy Sci., 91(4): 1497-1509.

14. Newbold, C.J., Wallace, R.J. and Cintosh, F.M. (1996) Mode of action of the yeast Saccharomyces cerevisiae as a feed additive for ruminants. Br. J. Nutr., 76(2): 249-261.

15. Chaucheayas-Durand, F., Walker, N.D. and Ach, A.B. (2008) Effects of active dry yeasts on the rumen microbial ecosystem: Past, present and future. Anim. Feed Sci. Tech., 145(1-4): 5-26.

16. Jonova, S., Ilgaza, A., Grinfelde, I. and Zolovs, M. (2018b) Impact of the flour of Jerusalem artichoke on the production of methane from carbon dioxide and growth in calves. Vet. World, 11(11): 1532-1538.

17. Dunn, O.J. (1964) Multiple comparisons using rank sums. Technometrics, 6(3): 241-252.

18. Miguel, J.C., Rodriguez-Zas, S.L. and Pettigrew, J.E. (2004) Efficacy of a mannan oligosaccharide (Bio-Mos®) for improving nursery pig performance. J. Swine Health Prod., 12(6): 296-307.

19. Tang, Z.R., Yin, Y.L., Nyachoti, C.M., Huang, R.L., Li, T.J., Yang, C., Yang, X.J., Gong, J., Peng, J., Qi, D.S., Xing, J.J., Sun, Z.H. and Fan, M.Z. (2005) Effect of dietary supplementation of chitosan and galacto-mannan-oligosaccharide on serum parameters and the insulin-like growth factor-I mRNA expression in early-weaned piglets. Domest. Anim. Endocrinol., 28(4): 430-441.

20. Król, B. (2011) Effect of mannan oligosaccharides, inulin and yeast nucleotides added to calf milk replacer on rumen microflora, level of serum immunoglobulin and health condition of calves. Electron. J. Pol. Agric. Univ., 14(2): 1-18.

21. Lesmeister, K.E., Heinrichs, A.J. and Gabler, M.T. (2004) Effects of supplemental yeast (Saccharomyces cerevisiae) culture on rumen development, growth characteristics, and blood parameters in neonatal dairy calves. J. Dairy Sci., 87(6): 1832-1839.

22. Roodposhti, P.M. and Dabiri, N. (2012) Effects of probiotic and prebiotic on average daily gain, fecal shedding of Escherichia Coli, and immune system status in newborn female calves. Asian Aust. J. Anim. Sci., 25(9): 1255-1261.

23. Wang, L., Feng, Y., Zhang, X. and Wu, G. (2019) Effect of probiotic Lactobacillus reuteri XC1 coexpressing endo-glucanase and phytase on intestinal pH and morphology, carcass characteristics, meat quality, and serum biochemical indexes of broiler chickens. R. Bras. Zootec., 48(1): 20180273.

24. Konca, Y., Kirkpinar, F. and Mert, S. (2009) Effects of mannan-oligosaccharides and live yeast in diets on the calf, cut yields, meat composition and colour of finishing Turkeys. Asian Aust. J. Anim. Sci., 22(4): 550-556.

25. Raghebian, M., Dabiri, N., Yazdi, A.B., Bahrami, M.J., Shomeyzi, J., Raghebian, A. and Hatami, P. (2007) Probiotic effect on meat quality and carcass parameters of Iranian Zandi lambs. J. Livestock Sci., 8(1): 163-168.

26. Giader, K.P., Shinde, A.K., Soren, N.M. and Karim, S.A. (2014) Effect of different levels of Lactobacillus acidophilus culture on carcass traits and meat quality of Malpura lambs. Rumin. Sci., 3(2): 229-234.

27. Tufarelli, V., Crovace, A.M., Rossi, G. and Laudadio, V. (2017) Effect of a dietary probiotic blend on performance, blood characteristics, meat quality and faecal microbial shedding in growing-finish pigs. S. Afr., 47(6): 875-882.

28. Lynch, H.A. and Martín, S.A. (2002) Effects of Saccharomyces cerevisiae culture and Saccharomyces cerevisiae live cells on in vitro mixed ruminal microorganism fermentation. J. Dairy Sci., 85(10): 2603-2608.

29. Mwenya, B., Santoso, B., Sar, C., Gamo, Y., Kobayashi, T., Arai, I. and Takahashi, J. (2004) Effects of including β-1,4-galacto-oligosaccharides, lactic acid bacteria or yeast culture on methanogenesis as well as energy and nitrogen metabolism in sheep. Anim. Feed Sci. Technol., 115(3-4): 313-326.

30. Frumholz, P.P., Newbold, C.J. and Wallace, R.J. (1989) Influence of Aspergillus oryzae fermentation extract on the fermentation of a basal ration in the rumen simulation technique (Rusitec). J. Agric. Sci., 113(1): 169-112.

31. Hernández, A., Kohlif, A.E., Elghandour, M.M.M., Camacho, L.M., Cipriano, M.M., Salem, A.Z.M., Cruz, H. and Ugbugo, E.A. (2017) Effectiveness of xylanase and Saccharomyces cerevisiae as feed additives on gas emissions from agricultural calf farms. J. Clean. Prod., 148(148): 616-623.

32. Takahashi, J., Chaudhry, A.S., Beneke, R.G. and Young, B.A. (1997) Modification of methane emission in sheep by cytokine and a microbial preparation. Sci. Total Environ., 204(2): 117-123.

33. Jonova, S., Ilgaza, A., Grinfelde, I. and Zolovs, M. (2018a) Impact of Inulin on Production of Methane, Carbon Dioxide and Gastrointestinal Canal Functionality in Calves. Proceedings of the 24th International Scientific Conference Research for Rural Development, Jelgava, Latvia. 264-270.

34. Guo, Y.Q., Hu, W.L. and Liu, J.X. (2005) Methanogens and manipulation of methane production in the rumen. Wei Sheng Wu Xue Bao, 45(1): 145-148.

35. Yanagita, K., Kamagata, Y., Kawaharasaki, M., Suzuki, T., Nakamura, Y. and Minato, H. (2000) Phylogenetic analysis of methanogens in sheep rumen ecosystem and detection of Methanomicrobium mobile by fluorescence in situ hybridization. Biosci. Biotech. Bioch., 64(8): 1737-1742.

36. Henderson, G., Cox, F., Ganesh, S., Jonker, A., Young, W. and Janssen, P.H. (2015) Rumen microbial community

Available at www.veterinaryworld.org/Vol.13/June-2020/1.pdf
composition varies with diet and host, but a core microbiome is found across a wide geographical range. *Sci. Rep.*, 5(1): 14567.

37. Leahy, S., Kelly, W., Ronimus, R., Wedlock, N., Altermann, E. and Attwood, G. (2013) Genome sequencing of rumen Bacteria and archaea and its application to methane mitigation strategies. *Animal*, 7(2): 235-243.

38. Wright, A.D.G., Auckland, C.H. and Lynn, D.H. (2007) Molecular diversity of methanogens in feedlot cattle from Ontario and Prince Edward Island, Canada. *Appl. Environ. Microbiol.*, 73(13): 4206-4210.

39. Whitford, M.F., Teather, R.M. and Forster, R.J. (2001) Phylogenetic analysis of methanogens from the bovine rumen. *BMC Microbiol.*, 1(1): 1-5.

40. Carberry, C.A., Waters, S.M., Kenny, D.A. and Creevey, C.J. (2014) Rumen methanogenic genotypes differ in abundance according to host residual feed intake phenotype and diet type. *Appl. Environ. Microbiol.*, 80(2): 586-594.

41. Danielsson, R., Schnürer, A., Arthursen, V. and Bertilsson, J. (2012) Methanogenic population and CH\(_4\) production in Swedish dairy cows fed different levels of forage. *Appl. Environ. Microbiol.*, 78(17): 6172-6179.

42. Danielsson, R., Werner-Omagic, A., Ramin, M., Schnürer, A., Grinari, M., Dicksved, J. and Bertilsson, J. (2014) Effects on enteric methane production and bacterial and archael communities by the addition of cashew nut shell extract or glycerol-an in vitro evaluation. *J. Dairy Sci.*, 97(9): 5729-5741.

43. King, E.E., Smith, R.P., St-Pierre, B. and Wright, A.D.G. (2011) Differences in the rumen methanogen populations of lactating jersey and holstein dairy cows under the same diet regimen. *Appl. Environ. Microbiol.*, 77(16): 5682-5687.

44. Alugongo, G.M., Xiao, J., Wu, Z., Li, S., Wang, Y. and Cao, Z. (2017) Review: Utilization of yeast of *Saccharomyces cerevisiae* origin in artificially raised calves. *J. Anim. Sci. Biotechnol.*, 8(1): 34.

45. Ogunade, I.M., Lay, J., Andries, K., McManus, C.J. and Bebe, F. (2019) Effects of live yeast on differential genetic and functional attributes of rumen microbiota in beef cattle. *J. Anim. Sci. Biotechnol.*, 10(1): 68.

46. Ding, G., Chang, Y., Zhao, L., Zhou, Z., Ren, L. and Meng, Q. (2014) Effect of *Saccharomyces cerevisiae* on alfalfa nutrient degradation characteristics and rumen microbial populations of steers fed diets with different concentrate-to-forage ratios. *J. Anim. Sci. Biotechnol.*, 5(1): 24.

47. Galindo, J., Marrero, Y., González, N., Sosa, A., Miranda, A.L., Aldana, A.I., Moreira, O., Bocourt, R., Delgado, D., Torres, V., Sarduy, L. and Noda, A. (2010) Effect of preparations with the viable yeasts *Saccharomyces cerevisiae* and LEVICA-25 on methanogens and in vitro ruminal methanogenesis. *Cuban J. Agric. Sci.*, 44(3): 267-272.

**********