Studying the possibilities to reduce methane emission in dairy cattle by adding Product X to the diet.

Onderzoek naar de mogelijkheden van de reductie van methaanemissie bij melkvee door gebruik van Product X via het voerspoor

Applied by:
Orffa Additives B.V.
CONO Kaasmakers

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Contents

1. Summary

With Product X, Orffa Additives BV (an European feed additives supplier) and Cono Kaasmaker (a Dutch dairy cooperative) are searching for a practical feed application to reduce methane emissions (>10%) in dairy cattle. In this study three *in vitro* experiments were conducted to test the characteristics of Product X.
The experiments were performed to test the cumulative gas, methane production (kinetics) and the fermentation end-products. It became clear that Product X is able to change rumen fermentation characteristics significantly. In the experiments Product X showed higher volatile fatty acids (VFA) levels and differences in methane production kinetics. It seems that the production of methane is delayed and the % of methane produced per total amount of gas is decreasing over weeks. This fact is especially of interest in live animals. Time feed remains in the rumen is relative short compared with this *in vitro* trial. With these results it also became clear that after six weeks of Product X administration there still is an effect on rumen fermentation and no signs of adaptation were found.
A reduction of 10% means a potential reduction of 9522 MT CO2-eq per year when only the members of Cono are taken into account (e.g. 37500 lactating animals). On national level this means 0.58 Mton CO2-eq reduction per year.
Reducing methane emissions is also improving feed efficiency. This means more milk for the same costs. It could be an option that Cono will implement an incentive to stimulate farmers to reduce methane emissions in their Caring Dairy program.

Key words: rumen fermentation, methane reduction, feed additive.

2. Samenvatting

Met het voederadditief Product X, zoeken Orffa Additives BV en Cono Kaasmakers naar een praktische toepassing om methaan emissie te reduceren (>10%). In deze studie zijn drie *in vitro* experimenten uitgevoerd om de effecten van het product Product X. Tijdens deze testen is naast cumulatieve gas en methaan productie(karakteristieken) de fermentatie eindproducten gemeten. Uit de proeven kwam duidelijk naar voren dat Product X in staat is om pensfermentatie significant te beïnvloeden. In de verschillende experimenten liet Product X naast hogere vluchtige vetzuur waarden, verschillen zien in methaan productie parameters. De productie van methaan wordt vertraagd en het % methaan van de totale hoeveelheid gas neemt af. Dit gegeven is zeker van belang in levende dieren. Zeker als de verblijftijd van voeders in de pens meegenomen wordt, in verhouding tot deze *in vitro* studie. Uit de gegevens wordt duidelijk dat na zes weken Product X verstrekking er nog steeds een effect is of pensfermentatie en er geen adaptatie optreed.
Een verlaging van methaan emissie van 10% betekent een potentiële reductie van 9522 MT CO2-equivalenten per jaar als alleen de leden van Cono (37500 lacterende melkkoeien). Op landelijke schaal betekent dit een potentiële reductie van 0.58 Mton CO2-eq per jaar.
Het verlagen van de methaan emissie heeft ook een effect op de voederconversie van het dier. Dit betekent meer melk voor minder kosten. Het is een mogelijkheid dat Cono Kaasmakers een stimuleringsregeling implementeert om het gebruik van methaan emissie reducerende maatregelen te stimuleren in haar Caring Dairy Programma.

Trefwoorden: pensfermentatie, methaan reductie, voederadditief.

3. Introduction

3.1 problem statement

The agriculture policy nowadays is focused on reduction of factors contributing to global warming. Main focus is reduction of the so-called greenhouse gasses (GHG). The main GHGs are: carbon dioxide (CO2), methane (CH4), nitrous oxide (N2O) and fluorinated gases (F-gasses). In 2007, CO2 equivalent emission from agriculture contributed 8.8% to the total emissions in the Netherlands. The agriculture sector is the largest source of CH4 and N2O emissions, contributing 53% and 61% respectively to the national emission total. Enteric fermentation is the main source of CH4 emissions, of which 90% derives from cattle (Van der Maas *et al.*, 2009). On average, cattle loses 6%, ranging from 2% to 12%, of their ingested energy as eructated methane (Johnson and Johnson, 1995).

In the rumen of ruminants, microbes live in symbiosis with their host. These microbes provide the animal with a source of microbial protein for production. Although microbes are able to use non protein nitrogen for growth, microbial protein does not always fulfil the requirement of high producing animals. Feeding additional protein can be costly and inefficient, which can result in higher excretion of nitrogen in the form of urea if not provided in a balanced way (Benchaar *et al.*, 2008a).

Because of environmental issues and the desire to improve the efficiency of nutrient utilization of the animal, evaluating methods to manipulate and improve efficiency of gastrointestinal micro flora in livestock is currently gaining more interest.

3.2 Objectives

With Product X, Orffa and Cono are searching for a practical feed application to reduce methane emissions with >10%. The aim of this project was to explore the full potential and mode of action of this product on methane reduction.

In this study Product X was in vitro tested on its potential to change methane production (kinetics) and VFA production.

3.3 Partners and other parties

This project is a cooperation between Orffa additives BV and Coöperatieve Zuivelonderneming Cono BA. Orffa is responsible for the knowledge within this project and Cono for the more practical aspects. As a third party the WUR – Animal nutrition Group was responsible for carrying out the in vitro gas production trials.

Cono Kaasmakers has a strong partnership with Ben&Jerry's Ice (USA). The Caring Dairy Project performed by Cono Kaasmakers is co-developed with Ben&Jerry's Ice. Therefore Ben&Jerry's Ice are also involved in the project, which enables a potential solution to be extrapolated on a bigger scale than Dutch dairy industry.

4. Materials en Methods

Three *in vitro* experiments were conducted to test the characteristics of product X. All three experiments were conducted by the WUR – Animal nutrition Group.
With these tests the cumulative gas, methane production kinetics and the fermentation end-products were measured. With these experiments Product X was tested on its potential to affect methane production and rumen fermentation.
For this project three fistulated non-lactating Holstein-Friesian dairy cows were used. The diet contained *ad libitum* grass silage. For the total experimental period the cows received Product X. The cows were used as rumen fluid donors for the *in vitro* trials.

4.1 Experimental design

4.1.1 Experiment 1: week 0

An *in vitro* gas production experiment was conducted to test the gas and methane production characteristics of Product X. After 72 hours volatile fatty acids (VFA) were measured to test effects on rumen fermentation. Product X was tested in duplicate bottles at levels 0 (control), 30, 150, 300, 1500 and 3000 ppm. Rumen fluid used in this experiment was obtained from de cows at day 0 of the experimental period i.e. cows had not been exposed to Product X.

4.1.2 Experiment 2: week 0 and 4

This *in vitro* gas production experiment was conducted to test the gas and methane production characteristics of Product X comparing week 0 and week 4 of adaptation to Product X. After 72 hours VFA were measured to test effects on rumen fermentation. Product X was tested in duplicate bottles at levels 0 (control), 30, 300, and 3000 ppm. Rumen fluid used in his experiment was obtained from cows at week 0 and after 4 weeks of receiving a daily dose of Product X.

4.1.3 Experiment 3: Substrates week 0, 2, 4 and 6

This *in vitro* gas production experiment was conducted to test the gas and methane production characteristics of two types of grass silage and two types of maize silage. In this experiment rumen fluid is obtained from the cows at 0, 2, 4 and 6 weeks of receiving Product X. After 72 hours VFA were measured to test effects on rumen fermentation. All four substrates were tested in triplicate bottles per time unit (week 0, 2, 4 and 6).

4.2 Measurements

4.2.1 Gas production measurements

Rumen fluid was collected from all three cows early in the morning before feeding as described by Cone et al. (1996). The fluid was transported in pre-warmed, insulated and with CO_2 filled flasks. In the laboratory the fluids was squeezed through two layers of cheesecloth and mixed with a pre-prepared buffer solution (2:1 buffer:rumen fluid). The buffer solution is made from the following components: trace elements ($CaCL_2.2H_2O$, $MnCL_2.4H_2O$, $CoCl_2.6H_2O$, $FeCL_3.6H_2O$), macro minerals (Na_2HPO_4, KH_2PO_4, $MgSO_4.7H_2O$), buffer solution ($NaHCO_3$, NH_4HCO_3), redox indicator (resazurin) and reducing agent (NaOH, $Na_2S.9H_2O$).
In 300 mL bottles 0,5 gram substrate and 60 mL buffered rumen fluid was added. In both experiments, grass silage was used as substrate and different levels of Product X were added. In each duplicate and/or triplicate series one blank (buffered rumen fluid without additive, with substrate) was added. The bottles were placed in pre-warmed shaking water baths at

39°C shaking at 40 movements per minute (figure 4.1). All steps taken in the laboratory, until the moment the bottles were placed in the water baths were done under continuous flushing with CO_2. This is done to unsure survivability of the anaerobic rumen microbes.
The bottles were connected to an automatic system for measuring the produced gas. The system used is described by Cone *et al.* (1996). Data was analysed and gas production curves were fitted using a biphasic model (Groot *et al.,* 1996).

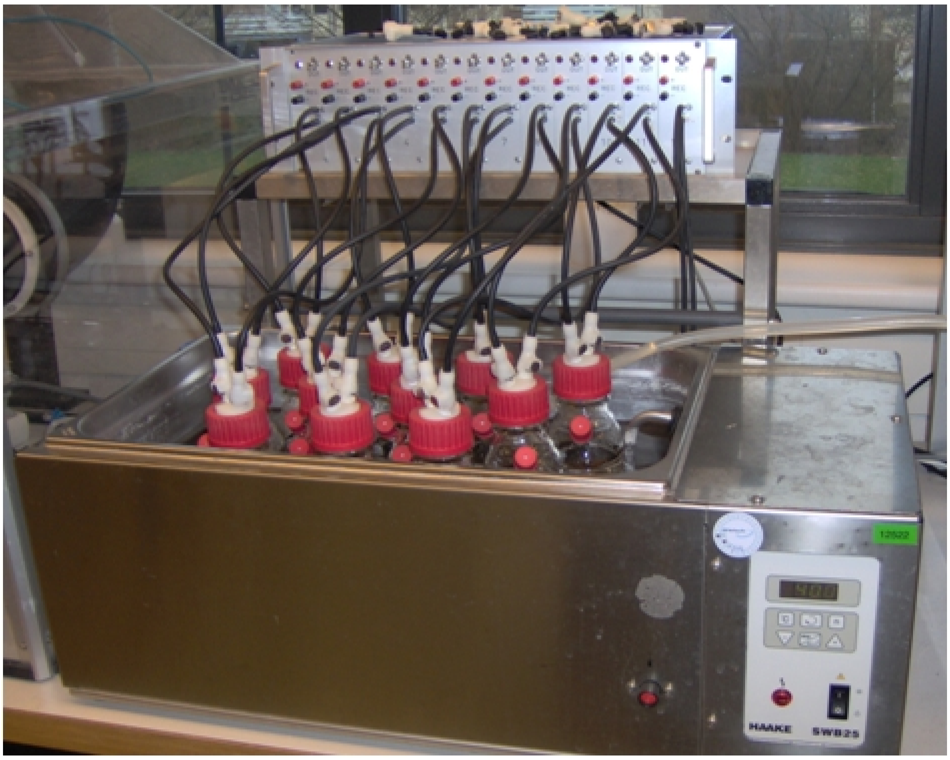

Figure 4.1: View of the experimental installation.

4.2.2 Methane production measurements

The bottles from the gas production test (4.2.1) were equipped with an additional screw cap fitted with a membrane suited for needle puncturing. Using an air tight syringe with fixed needle, 10 µL of gas was taken from each bottle headspace to test for methane. The samples were tested on methane with a gas chromatograph. Data on methane production curves were fitted with a model as describe by Groot *et al., (*1996).

4.2.3 VFA

For VFA measurements fermentation fluid was collected and analysed using a gas chromatograph. VFA recognized: acetic acid, propionic acid, butyric acid, iso-butyric acid, valeric acid and iso-valeric acid. Total VFA is sum of these volatile fatty acids.

5. Results

5.1 Experiment 1: week 0

In this experiment Product X was tested *in vitro* on its gas and methane (CH_4) production and kinetics. Volatile fatty acids (VFA) was measured after 72 hours. Doses of 0 (control), 30, 150, 300, 1500, 3000 ppm were tested. Figure 5.1 shows the curve fits for the methane production of Product X. All levels of Product X showed comparable gas production curves. VFA levels had a tendency to increase after 72 hours for higher levels of Product X.

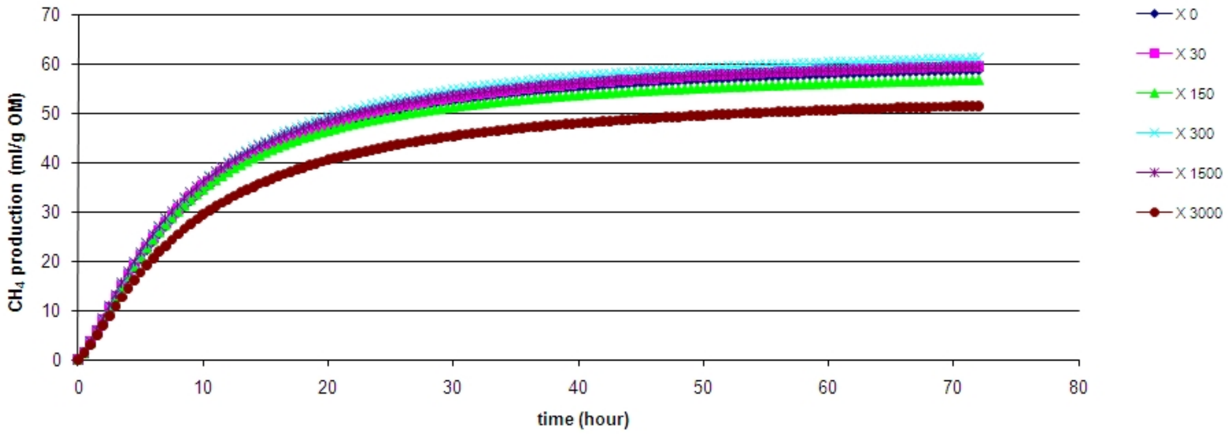

Figure 5.1: CH_4 production for different levels (0, 30, 150, 300, 1500, 3000 ppm) of product X.

5.2 Experiment 2: week 0 and 4

In two different runs product X was tested *in vitro* on its gas and methane production and kinetics. Volatile fatty acids (VFA) were measured after 72 hours. Product X was tested with doses of 0 (control), 30, 300, 3000 ppm. For this experiment rumen fluid is used from cows at 0 and 4 weeks of administration with product X.

Total gas and methane production did not change significantly between the different doses. At week 4 al doses tested showed a numerical increase in total VFA concentration at week 4. This is shown in figure 5.2.

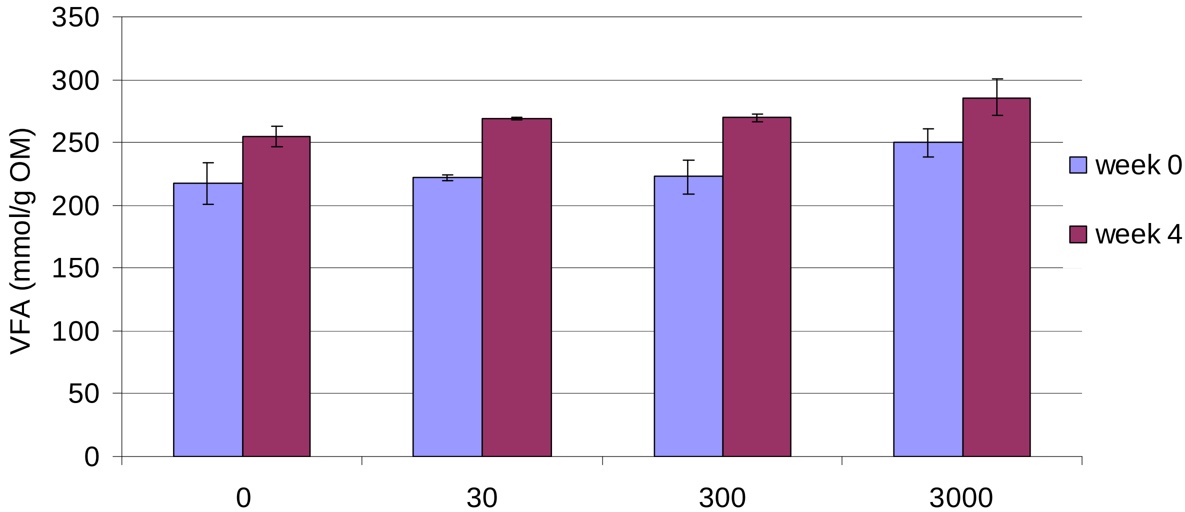

Figure 5.2: Total volatile fatty acids (VFA) concentrations of four concentrations of product X in two different runs at 0 and 4 weeks.

5.3 Experiment 3: In vitro substrates week 0, 2, 4 and 6

In this experiment two different sources of maize silage and two different sources of grass silage were tested *in vitro* on their gas and CH_4 production and kinetics. Also VFA's were measured. All four substrates were tested in four runs with rumen fluid from cows after 0, 2, 4 and 6 weeks of adaptation to Product X. Two maize samples and two grass samples were selected on their differences in digestibility; sample 1 was higher digestible then number 2.

5.3.1 Gas and methane kinetics

No significant differences were found for total gas production and production kinetics between the four runs for each substrate. Total CH_4 production were the same for all four runs, but CH_4 kinetics were different. Figure 5.3 shows the percentage of CH_4 of the produced gas OMCV for maize 1 per run. The trend becomes visible of a lower percentage of CH_4 of total gas measured as Organic Matter Cumulative Volume (OMCV) produced per run at approximate 5 hours. The Figure also shows that the first gas produced was high in CH_4 and that the percentage of CH_4 was going down over time, suggesting production of other gasses is exceeding that of methane production.

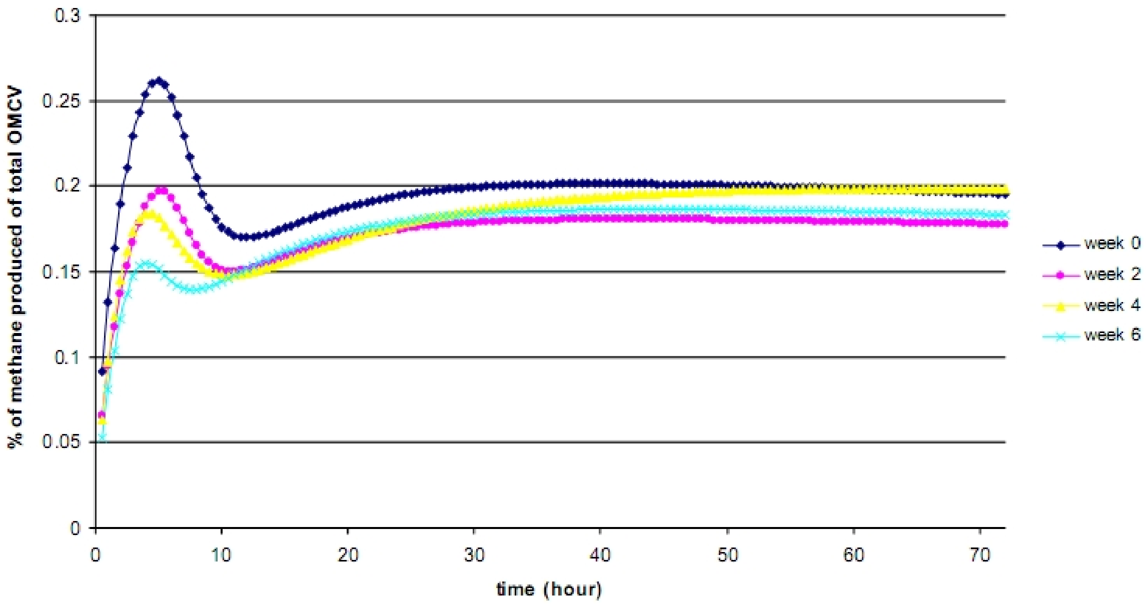

Figure 5.3: Percentage of methane produced of total gas with Maize 1 in four different runs at week 0, 2, 4 and 6 weeks.

5.3.2 VFA

Total volatile fatty acids (TVFA) levels were increasing with runs for all four substrates, as can be seen in Figure 5.4. For grass silage 1, grass silage 2 and maize silage 2 TVFA was in the first run from week 0, significant lower then the last run of week 6.

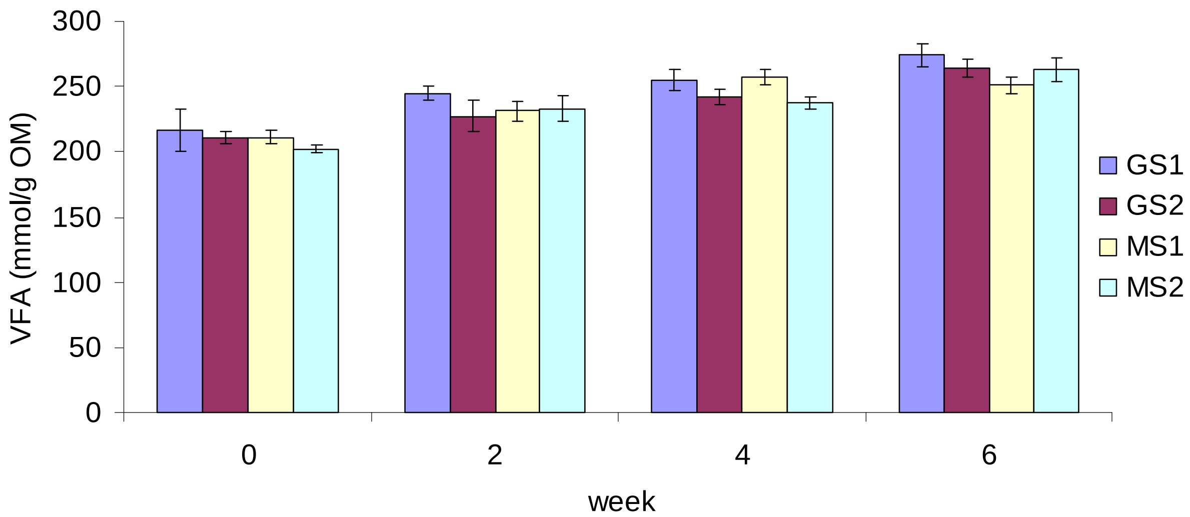

Figure 5.4: Total volatile fatty acids (VFA) concentrations of four substrates in four different runs at 0, 2, 4 and 6 weeks.

5.4 Environmental aspects

The results in this report are based on in vitro/lab experiments. Although no live animals are used, they give a clear indication on the methane reduction potential of Product X. The results from this project show that Product X is able to change methane production kinetics. Results should be confirmed in climate respiration chambers with live animals, but a methane emission reduction of 10% seems possible.

With a reduction potential the following calculation can be made. When cows produce on average 362 gram CH4 per day (Perdok et al, 2007) a reduction of 10% will mean 36.2 grams per cow per day. In CO2 eq: 36.2 x 23 = 832.6 gram CO2-eq/cow/day. It is assumed the cows receive the additive during lactation, this means during 305 days per year: 0.25 MT CO2/cow/year.
When only the members of Cono are taken into account (e.g. 37500 lactating animals) the reduction potential will be 9522 MT CO2-eq per year.
On national level the methane emission from the rumen of dairy is 5,8 Mton CO2-eq/year. A 10% reduction means 0.58 Mton CO2-eq reduction per year.

5.5 Economical aspects

Methane reduction by the use of feed additives requires no additional investments for dairy farmers. The additive can easily be distributed through the feed.
For the following it is assumed that Product X is effective with a dose of 5 gram per day. The costs of Product X are set on €12,- / kg, delivered on farm through concentrate feed.
The costs for an average farm with 75 cows in lactation for 305 days are: 5gr x 305 x 75 x €12 = € 1372.50. It is an option that costs for the use of Product X will partially be covered by Cono through implementation of an incentive for the use of methane reducing arrangements. It can be assumed that milk production will rise with 1 kg/cow/day by the use of Product X. Also several parameters measured during these in vitro trials showed a positive trend for parameters defining an increased milk production. For a farm with 75 lactating animals this will be: 75x305x1.0= 22875 litre/year. Because of milk quota, a higher production will only pay off because of the use of fewer animals. With a production of 9000 litre/cow/year 2.5 cows (3.3%) less are needed to produce the same amount of milk. This will reduce the non-fixed costs. According to the LEI the non-fixed costs are +/- € 10/100 litre of

milk. Lowering the costs for an average farm of 75 cows means a reduction of:
22875x€10/100litre = € 2287.50.
The benefits from operations is € 1372.50 + 2287.50 = 915/3660 per dairy farm/year. For this calculation it is assumed the costs for the use of Product X (€ 1372.50) is covered by Cono via an incentive for the use of methane reducing arrangements. These calculated incentives are lower than the ones given in other clauses of Caring Dairy Program such as incentives for grazing, so can be considered a realistic option.

5.5.1 Cost effectiveness of Product X

The methane emission reduction per cow is 0.25MT CO2-eq/cow/day. For an average farm with 75 lactating dairy cows this is: 19.05 MT CO2-eq/year.

The cost effectiveness = (cost of capital+ operational costs – revenues) / total yearly emission reduction. = (0 + 1372.50 – 3660) / 19.05 = - € 120,- / MT CO2-eq.

5.6 Application of Product X

A successful tool for reducing methane emission can be implemented easily through the feed. Within Orffa Additives the first focus are dairy cows, but can easily be transferred to other species like beef and sheep.
Because of the strong international character of Orffa this project can be scaled up to an international level. Also with the cooperation of a strong brand name like Ben&Jerrys a large group of dairy's can be reached and convinced of taking methane reducing measurements.

5.7 Non technical points of interest

The application of a product to reduce methane emissions possible needs registration. This product should be safe to use, although registration might be time consuming.
Cono is leading in sustainable dairy production. Through their network methane emission reducing measurements are easily implemented. With the use of workshops farmers can be informed on the use of Product X.

5.8 Next steps

With Product X, Orffa and Cono are jointly searching for a practical application to reduce methane emissions with >10%. The outcome of this project gave good indications that Product X is able to change rumen fermentation characteristics and reduce methane emissions. During the in vitro experiments it became clear that methane production kinetics changed by Product X.
Therefore in vivo confirmation with climate respiration chambers is needed. With the use of these climate respiration chambers and PCR analyses the mode of action of Product X is explored. PCR analyses can be used to study a possible change in rumen microbial population, thereby explaining which type of organism is responsible for lower methane production. Previous studies from literature focused on the link of methanogenic microbes with rumen protozoa. It is known that part of the methane producing microbes live in symbiosis with protozoa. This is only part of the methane producing microbes; numerous protozoa do not provide clear information on methane production. More knowledge is needed to understand the complete process of methanogens reduction. Therefore, our focus is not only the microbes linked to protozoa, but to create a complete microbial profile with PCR. In doing so we can gain knowledge on the effects in the rumen and gain insight in the mode of action.

6. Extensive summary and conclusions

The rumen of ruminants is producing methane as a waste product of feed fermentation. This raises the desire to reduce the methane production not only because of environmental reasons but also improving the efficiency of nutrient utilization of the animal.

With Product X, Orffa Additives and Cono Cheese makers are searching for a practical feed application to reduce methane emissions with >10%. The aim of this project was to explore the full potential and mode of action of Product X on methane reduction.
In this study the product was in vitro tested on its potential to change methane production (kinetics) and VFA production.

In this study three *in vitro* experiments were conducted to test the characteristics of Product X. All three experiments were conducted by the WUR – Animal nutrition Group.
With these tests the cumulative gas, methane production kinetics and the fermentation end-products were measured. With these experiments Product X was tested on its potential to affect methane production and rumen fermentation.
Three fistulated non-lactating dairy cows were used as rumen fluid donors for the *in vitro* experiments. The cows received Product X during 6 weeks. The following 3 experiments where conducted:
Experiment 1: Testing different levels of Product X. Methane and gas production (kinetics) and rumen fermentation products were taken into account. Rumen fluid used in this test was obtained from the cows at day zero of the experimental period i.e. cows had not been exposed to Product X.
Eperiment 2: Different levels of Product X were tested comparing week 0 and 4 of adaptation to Product X. Rumen fluid used in his experiment was obtained at week 0 and after 4 weeks of receiving daily doses of Product X.
Experiment 3: Two types of grass silage and two types of maize silage were tested on gas/methane production and fermentation products. In this experiment rumen fluid is obtained from the cows after 0, 2, 4 and 6 weeks of receiving Product X.

The results from this study show that Product X is able to change rumen fermentation characteristics significantly. The final total amount of methane produced did not change necessary, more interesting are production kinetics. It seems that the production of methane is delayed. This is of interest if the time feed is staying in the rumen (live animals) is taken into account. The production of total VFA was higher with comparable branched chain VFA levels for Product X. This means the proportion of branched chain VFA was reduced. From the results of experiment 3 it becomes clear that after approx 5 hours the amount of methane produced per % of total gas is decreasing over weeks. With these results it becomes clear that after six weeks of Product X rumen microbes still show an effect on Product X and no signs of adaptations were found. This is also confirmed by the total VFA production, which is increasing over time. With all four substrates the total VFA production remains higher, also after a period of 6 weeks.
To conclude these results, Product X clearly shows an effect on rumen fermentation and methane production kinetics.

Orffa and Cono are aiming at a 10% reduction of methane emission. A reduction of 10% means a potential reduction of 9522 MT CO2-eq per year when only the members of Cono

are taken into account (e.g. 37500 lactating animals). On national level the methane emission from the rumen of dairy is 5,8 Mton CO2-eq/year. A 10% reduction means 0.58 Mton CO2-eq reduction per year.
Reducing methane emissions is also improving feed efficiency of the cow. This means simply more milk for the same costs. It is an option that Cono will implement an incentive to stimulate farmers to reduce methane emissions.

The application of a product to reduce methane emissions is only possible with the correct registrations and should be safe for use in animal feed.
Cono is leading in sustainable dairy production. Through their network methane emission reducing measurements are easily implemented. With the use of workshops farmers can be informed on the use of Product X.

To confirm the methane reduction by Product X, however, in vivo confirmation is needed. The use of climate respiration chambers and PCR analyses are needed to gain more knowledge on the mode of action and to explore the full potential of Product X. In addition to this we are able to look for possible effects on production parameters and the most favourable practical dose. If successful, this project provides a solid scientific base for a successful feed additive able to reduce methane production in dairy cows.