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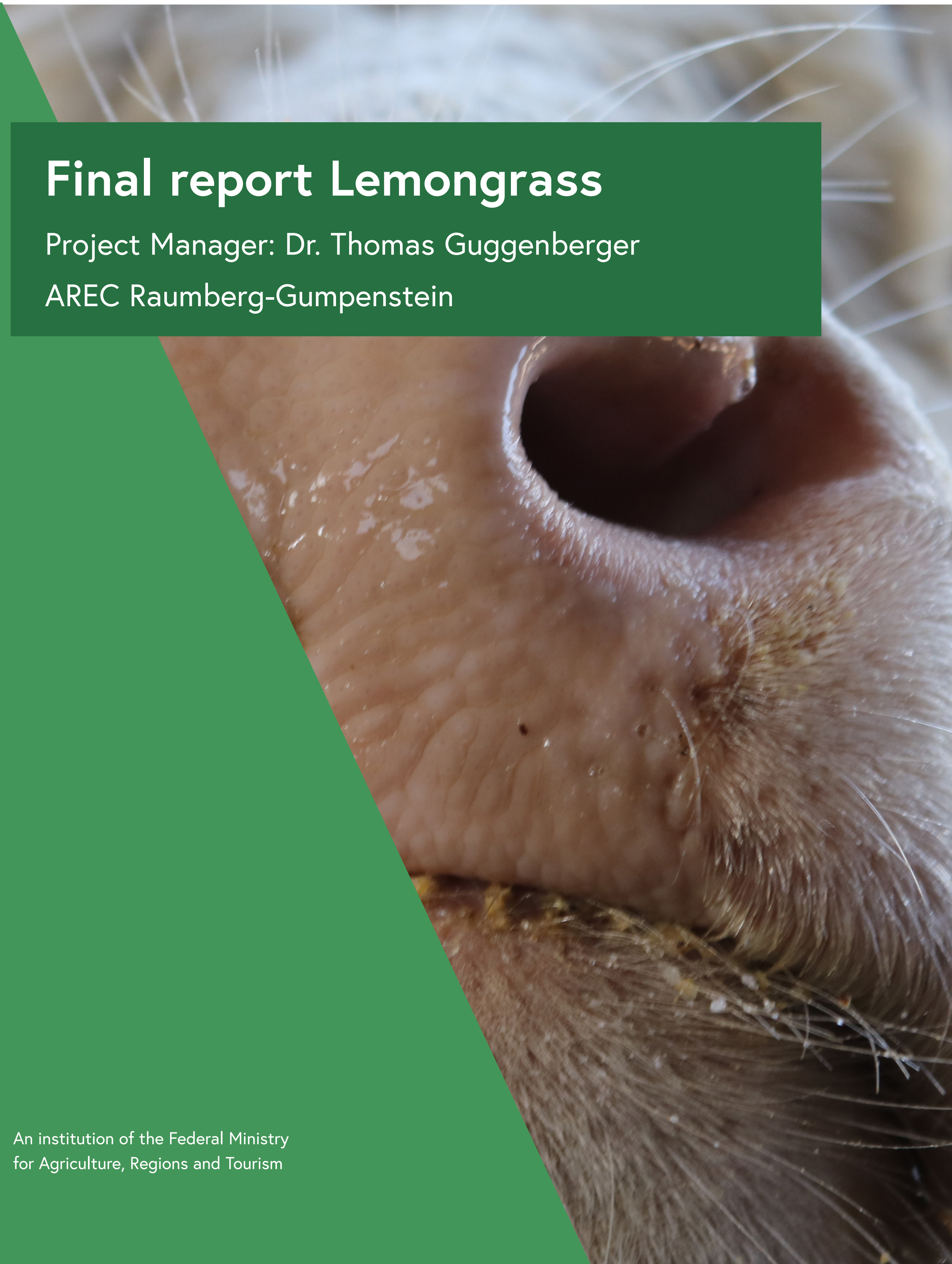

Final report Lemongrass

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An institution of the Federal Ministry
for Agriculture, Regions and Tourism

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Effect of 100 g lemongrass as feed supplement on methane concentration in the respiratory air of beef cattle

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Effect of 100 g lemongras as feed supplement on methane concentration in the respiratory air of beef cattle

Summary

Lemongrass: this natural feed contains more than 6 % condensed tannins, and can reduce methane emissions from cattle, according to existing studies from Mexico. On the initiative of the Marcher Fleischwerke, this possible effect was field-tested under Austrian conditions (high proportions of maize silage in the ration, good feed structure, supplemented with cereals and rapeseed meal). Eight stalls with six animals each were available for examination at the Schrammel test farm. Two comparable adjacent stalls formed a group whereby the animals (male cattle, 450 kg live weight, daily increases of 1,350 grams on average) in one stall were fed with an addition of 100 grams of lemongrass per animal per day. This quantity represents a ration ratio of between 1.7 and 1.2%, depending on age. In four three-week periods, each stall was fed twice with and twice without lemongrass. At the end of each period, the concentration of methane (CH_4) in the breath of cattle was measured. The measuring sensor Laser Methane mini (LMm) from the Tokyo Gas Engineering Solution was used for this purpose. In addition, 16 animals were equipped with ruminal boluses (PH Plus, Classic Plus) from smaXtec. These sensors detect processes in the rumen and provide information about activity and drinking behavior. All feeds used have been chemically tested for their ingredients and feed value.

Using the general linear model, the following results were obtained from the raw data:

Feeding 100 grams of lemongrass reduces CH_4 emissions in the breath by an average of 14.6%. The range of variations in the repeats were between 7.8 and 23.4%. The groups differed quite significantly.

There is unlikely to be a negative influence on the processes in the rumen at this amount of lemongrass. All results of the ruminal boluses are typical over this course of time and differences were not statistically significant.

The known methane-reducing effect of feeding lemongrass from the literature could also be confirmed under Austrian conditions.

Introduction

Gaseous emissions are a natural part of the metabolic activity of bacteria and protozoa. The resulting gases are specific to bacterial groups and have different effects on the organism and the environment. Ruminants are evolutionarily designed for a symbiosis with rumen and intestinal bacteria and could not use the complex carbohydrates of plants without their help. In particular, the degradation of stable cell walls requires a group of bacteria that break off hydrogen (H) from carbohydrates during their activities. Some of it is linked to carbon dioxide (CO_2) to form methane (CH_4), which escapes through the mouth or rectum. Biogenic CH_4 is a low-density odourless gas that does not cause any damage in the immediate vicinity of the animal. It is combustible in higher concentrations and is therefore used to generate energy in biogas plants. When CH_4 rises into the atmosphere, it contributes to global warming because of its effectiveness as a major greenhouse gas.

In the wake of global population development which has roughly quadrupled in the last 100 years, the global herd of ruminants has also grown sharply and is therefore rightly in the public debate across the world today. The driving force, however, is not the individual animal itself, but the total stock created by humanity. This has developed in different countries in completely different ways. In Austria, the cattle population is decreasing over the long-term. Since the stabilization of global population density is not foreseeable, the growth relationship with the global ruminant population must be weakened for climate protection, as well as the relationship between economic growth and the use of fossil energy. In practice, this means at least capping a globally permissible CH_4 emission from agriculture, which can only be achieved by changing the size of the herd or by alternative (technical or biogenic) solutions.

State of knowledge

In order to achieve a reduction in methane emissions from the microbial digestive processes in the rumen, it is necessary to clarify how methane is produced. The rumen ecosystem is made up of many different types of microorganisms. Roughly these can be placed into three groups: bacteria, archaebacteria as well as protozoa and fungi (BRADE and DISTL 2015a). Archaebacteria and protozoa are particularly important for the process of methane formation. Archaebacteria produce methane as a metabolic end product and live in close symbiosis with protozoa (BRADE and DISTL 2015b). Protozoa promote protein and cell wall degradation in the rumen and therefore contribute to the digestion of hard-to-degrade feed components. However, protozoa also reduce the efficiency of feeding, as they consume high-quality bacterial protein (protein or nitrogen loss) on the one hand and, on the other, through their symbiosis with archaebacteria, boost methane production (BRADE and DISTL 2015a). Methane-reducing measures are therefore also aimed at reducing protozoa.

Measures to reduce methane emissions from ruminant digestion

In a recent review article, BEAUCHEMIN et al. (2020) mentions the following three areas in which methane-reducing measures can be taken:

- Management and breeding
- Feeding
- Change in microbe composition and digestive processes in the rumen

In the field of management and breeding, increasing productivity and efficiency can contribute to lower methane emissions per unit of product produced. There are also international efforts to develop breeding values for the characteristic methane production (BRADE and DISTL 2015b, BEAUCHEMIN et al. 2020). Taken as a whole, reducing animal losses and extending the useful life of ruminants can also contribute to the reduction of methane emissions while maintaining production capacity (BRADE and DISTL 2015b). However, for this effect to occur, the total ruminant stock must also decrease.

In the field of feeding, a reduction in methane emissions can be achieved by increasing the starch content and reducing the fiber content in the ration (BRADE and DISTL 2015b). This is possible by increasing the proportion of maize silage and/or concentrated feed in the ration, or by improving the quality of the feed (JAYASUNDARA et al. 2016, BEAUCHEMIN et al. 2020). Another possibility is the use of feed fats (BRADE and DISTL 2015b, JAYASUNDARA et al. 2016, BEAUCHEMIN et al. 2020).

The microbe composition and digestive processes in the rumen can be influenced either by vaccination of the host animal and defaunation (removal of protozoa from the rumen) or by the use of feed additives (BRADE and DISTL 2015b). Numerous methane-reducing feed additives are now known. These include, among others, chemical inhibitors (e.g., 3-nitrooxypropanol), inorganic feed additives (e.g., nitrate), antibiotic active substances (e.g., monensin), organic acids (e.g., propionic acid precursors) and natural extracts (e.g., secondary plant ingredients, yeasts, algae) (BRADE and DISTL 2015b, JAYASUNDARA et al. 2016, BEAUCHEMIN et al. 2020)

Feed additives and their effect

The effect of methane-reducing feed additives is usually based on inhibition of the metabolic pathways of archaebacteria and protozoa. For archaebacteria to form methane, they need hydrogen (H_2). One approach of methane reduction is therefore to target substances in the rumen, which can also bind H_2. These include, for example, natural metabolic products of the rumen (e.g., propionate), but also inorganic feed additives, such as nitrate (BEAUCHEMIN et al. 2020). Another approach is to use feed with a high content of secondary plant ingredients (e.g., tannins, saponins). Saponins form complexes in cell membranes of protozoa, which lead to their death. Tannins form complexes with proteins and thus reduce the protein degradability and availability of protein for the microorganisms in the rumen (GOEL and MAKKAR 2012). The group of tannins includes numerous different substances, whereby a distinction can be made between hydrolyzed and condensed tannins, which differ in their mode of action. While hydrolyzed tannins inhibit the growth of methane-forming microorganisms, condensed tannins reduce the degradability of fiber components of the feed (GOEL and MAKKAR 2012, HRISTOV et al. 2013, MIN et al. 2020).

In addition to the mode of action, the extent of methane reduction also differs significantly between different tannins (BEAUCHEMIN et al. 2020, FAGUNDES et al. 2021). ROCA-FERNANDEZ et al. (2020) investigated rations of 50% grass and 50% legumes. The legumes differed in their content of condensed tannins (2.3 to 147.7 g/kg dry matter (DM)). The methane-reducing effect increased with increasing tannin content of legumes. However, the feeding of legumes with the highest tannin content led to a highly significant decrease in the digestibility of the ration. NIDERKORN et al. (2020) added 20 g/kg DM of sainfoin pellets, hazelnut shells and a mixture of both and also found different effects on methane production, although in all three cases significantly lower values were found compared to a base ration. The use of an acacia extract (containing condensed tannins) also resulted in a significant reduction in methane emissions from beef bulls, without significantly reducing the animals' performance. Only the digestibility of the neutral detergent fiber (NDF) of the feed decreased significantly (STAERFL et al. 2012). Mixing quebracho tree extract (containing condensed tannins) into a beef steer ration, on the other hand, had no methane-reducing effect (EBERT et al. 2017). In the trials by ABOAGYE et al. (2018) and ABOAGYE et al. (2019), the addition of 1.5 to 2.0% of an extract with a high content of hydrolyzed tannin into the ration of beef heifers also did not lead to a significant decrease in methane emissions per day and per kg of DM intake. However, 1.5% of a mixture of hydrolyzed and condensed tannins was found to have a significant methane-reducing effect (ABOAGYE et al. 2018). Lemongrass (60 g condensed tannins/kg DM) was fed to beef cattle in 2 trials by VÁZQUEZ-CARILLO et al. (2020). In the first trial, each beef cattle (390 kg live weight) received 100 grams of lemongrass per animal per day. In the second trial, the lemongrass was mixed with a proportion of 2%, 3% and 4% respectively and fed to the beef cattle (500 kg live weight) in the ration. In both trials, a significant effect of lemongrass was found.

Materials and methods

BK Cows Menu

Burger King International (BK) has selected lemongrass from the work of a research group of the Faculty of Veterinary Medicine and Animal Science, Autonomous University of the State of Mexico (VÁZQUEZ-CARILLO et al. 2020) in order to position a strategic development and marketing process in the media called „Burger King Cows Menu“ (BK 2020). BK recommends that producers add 100 g of dried, crushed lemongrass per animal per day and expects a significant reduction in CH_4 emissions based on research from Mexico. Beef cattle (Charolais x Brown Swiss) were used, as already described, in two different experimental approaches. Trial 1 was carried out with beef cattle weighing around 390 kg with a ration of 80.6% concentrated feed and 19.4% very fiber-rich basic feed (alfalfa hay and oat straw). In this trial not only lemongrass (Cymbopogon citratus), but also chamomile (Matricaria chamomilla) and garden cosmos (Cosmos bipinnatus) were studied. All groups received a constant amount of feed additive. In trial 2 only lemongrass was used, having had the strongest effect in trial 1. The added amount was now 2%, 3% and 4% of the ration, respectively. The overall ration was changed, with the proportion of fiber-rich forarge increased to 30.1%.

The CH_4 measurements were carried out with four animals in a Latin square and a trial period of 21 days in a respiration chamber. All production and emission data were available in both trials. The scientists were therefore able to calculate both, the load and the emission quantity per kg of dry matter intake, as well as the emission quantity per kg of daily increase for CH_4. In trial 1, lemongrass reduced the CH_4 load per day by 16.4% and the CH_4 emissions per kg of DM by 33.0%. CH_4 emissions per kg of daily gain were reduced by 22%. In trial 2, the addition of 2%, 3%, and 4% of lemongrass reduced the CH_4 load per day by 26.0%, 26.3%, and 15.3%. CH_4 emissions per kg of DM decreased by 12.0%, 15.5% and 0.5%, and those per kg of daily gain by 21.0%, 18.9% and 13.4% respectively. For later interpretation, it must be noted that because of the different measuring methods, the load can most likely be used as a comparative value. The expected value for a reduction is therefore between 16.4% (load test 1) and 26.0% (load group 2%, test 2).

From the point of view of a European research institution, the trial can be evaluated methodically and technically as conforming to the standard. The overall short duration of the trial and the small number of animals are typical for the complex investigations of gaseous emissions in respiratory chambers.

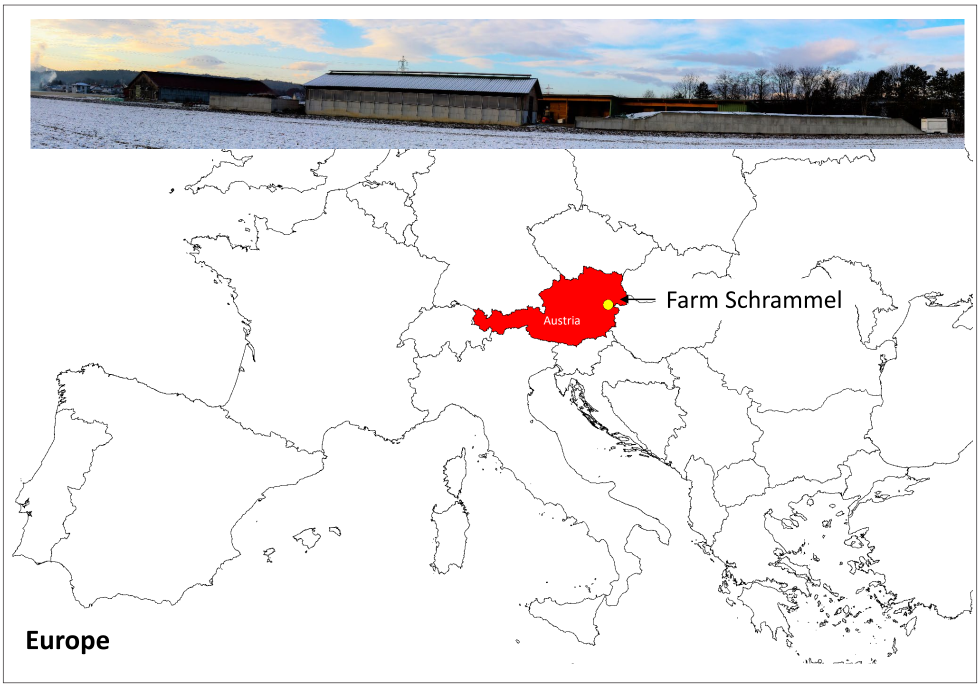
BK itself has published the initially observed effect and the recommendation to feed 100 g of lemongrass per animal per day throughout its partners across the world. In Austria, the Marcher Fleischwerke in Villach took up the idea and, together with AREC Raumberg-Gumpenstein, implemented a replication of the trial under Austrian conditions. Since the measurement of individual animals in the respiration chambers in Raumberg-Gumpenstein is only allowed for 4 days per year, no Latin square can be applied here. The chosen experimental design was therefore carried out on a farm as a field test.

Description of farm

The farm of the Schrammel family, 2625 Schwarzau am Steinfeld, Austria is located on the edge of the Eastern Alps at an altitude of 330 meters above sea level. Climatically, it has an average annual temperature of 8.6°C and an average annual precipitation of 600 mm. The Steinfeld is a large, almost flat gravel deposit in the southern region of the Vienna Basin formed during the Riss glaciation period. During average years, the farm achieves very good crop yields, but the site is also at risk of drought.

The Schrammel family manages 90 hectares of farmland with 16% permanent pasture (3-5 cuts per year), 27% silage maize, 33% field fodder, 16% cereals and 8% alfalfa. There are currently 65 dairy cows with their calves and 120 beef cattle on the farm. The average herd yield of milk production is 9,800 kg of milk per

Figure 1: Location of the farm



cow, per year. The fattening capacity of the beef cattle averages 1,300 grams per animal per day over several years (*Figure 1*).

Description of the trial

The fattening barn was built in 2015 using what is a common design in Austria. It is a wood and concrete construction spanned by a cantilever roof. Feeder stalls with slatted floors are located to the left and right of the drive-on feeding axis. Each stall was designed for 6 cattle. The feeding area is equipped with a safety gate to hold the animals. The feeding axis can be closed on each side with a sectional door. Roll curtains regulate the fresh air supply along the feeder stalls. Thanks to the relatively large volume of the building and the possibility of lateral ventilation, the barn has a favorable climate with sufficiently cool, fresh air during the trial period. A similar form is in the rearing barn.

In order to ensure that all selected fattening animals can fulfil the full test plan, stalls were selected where the average age was less than 400 days at the start of the trial. The two stalls with the youngest animals (235 and 264 days respectively) are located on the left side of the barn in *Figure 2*, with all others on the right. Each stall is equipped with 6 feeder bulls of the breed Fleckvieh. Stall 6 is missing an animal that had failed before the trial. The animals cannot be weighed in this stable, but due to the many years of experience in this system and a consistently high growth rate of an average of 1,350 g per animal per day, a number of parameters can be assumed via mathematical functions. This is based on the requirement standards issued by the Society for Nutritional Physiology (GfE 1995, 1999), the Gruber Table (LfL 2020) and the HBLFA's own work (STEINWIDDER et al. 2006). Using this method, the individual animals were assigned an estimated live weight (kg), an estimated feed intake (kg dry matter DM) and an estimated requirement for metabolizable energy (MJ ME) and protein

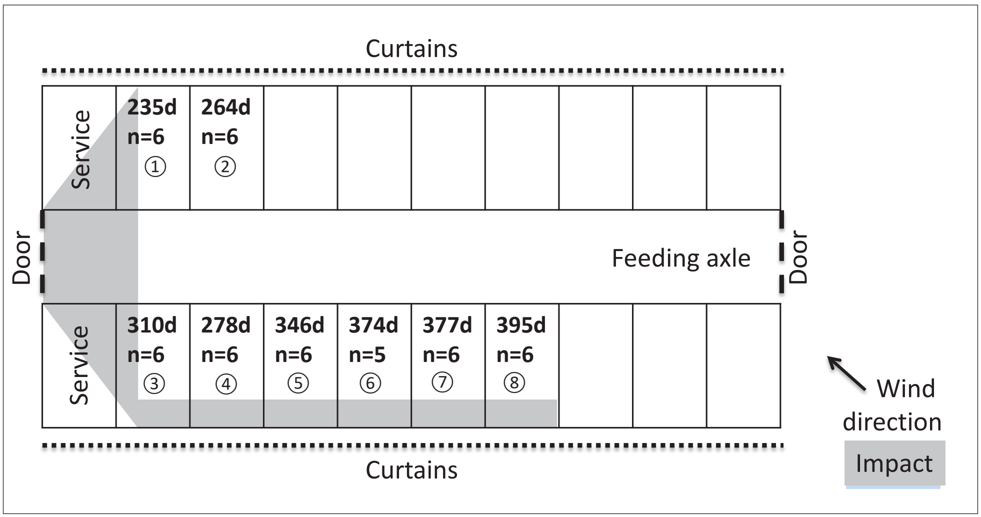


Figure 2: Floor plan of the fattening barn

Figure 3: Interior shots of the rearing barn

Figure 4: Interior shots of the fattening barn

(g crude protein XP) for each test day. The models used were readjusted with each repetition by weighing the amount of feed presented. This weighing was carried out via the weighing system of the mobile feed mixer. The feed residues were weighed manually.

Table 1: Feed and nutrient concentrations

Feed	Mixing ratio		Weender nutrients and fiber components											Energy	
			FM	XP	XL	XF	XA	XX	OM	NDF	ADF	ADL	NFC	ME	NEL
	% FM	% DM	g/kg DM											MJ / kg DM	
Corn silage	62.3	52.2	362	89	27	251	59	574	941	501	273	33	325	10.25	6.12
Brewer's grain silage	11.1	6.5	251	284	94	175	53	394	947	554	267	78	14	12.51	7.59
Grass silage	8.9	5.3	260	166	32	281	110	411	890	481	316	32	211	9.86	5.85
Rapeseed meal	5.3	11.0	890	399	25	131	77	368	923	319	221	107	180	11.99	7.31
Grain maize	4.4	9.1	880	106	45	26	17	806	983	120	30	6	712	13.26	8.39
Barley	4.4	9.1	880	124	27	57	27	765	973	216	63	12	606	12.84	8.08
Barley straw	2.7	5.3	850	45	12	435	60	448	940	785	455	18	98	6.62	3.65
Minerals	0.8	1.6	900												
TMR[1]	100	100	432	141	32	202	69	554	931	430	233	38	326	10.72	6.49
TMR[2]			432	139	34	218	77	532	923	461	263	42	289	10.84	6.56

[1] Mathematical result of the individual components
[2] Mean chemical analysis of the completely mixed DM
fresh mass (FM), dry mass (DM), crude protein (XP), crude fat (XL), crude fiber (XF), crude ash (XA), N-free extracts (XX), organic mass (OM), Neutral-Detergent Fiber (NDF), Acid-Detergent Fiber (ADF), Lignin (ADL), Non-fiber carbohydrates (NFC), metabolizable energy (ME), Net Energy Lactation (NEL)

At the beginning of the trial on 21.9.2020, a sample was taken from all feed materials, and the dry matter content, the Weender ingredients, the fiber components and the energy content (*in vitro* method ELOS) were examined in the chemical laboratory of AREC (*Table 1*). For each feed, the proportion of mixture is known, which is why the nutrient concentration of the mixture can be calculated from the feed. At each repetition, a sample was also taken from the total mixed ration submitted and examined just like the feed materials. In the last two rows of *Table 1*, the added results of the individual components are compared with the averages of the mixed samples. The deviations are small.

The ration in this trial differs very much in the composition of the individual components from the reference ration described. The high proportion of cereals used with the test animals in Mexico is tolerated only because very structurally rich feed was fed to them at the same time. Due to the high proportion of maize silage and the grass silage content, the ration already has a very balanced nutrient ratio and a sufficient structure for ruminants. The proportion of concentrated feed is around 31%. This enables feeding that is gentle on the rumen, as the pH value only experiences small fluctuations. The trial ration is less energy-efficient and more structurally rich than the ration of the reference test.

The lemongrass required for the trial was sourced from Natural Origins, Lozanne, France. The product is gently dried, chopped and bagged. It is comparable in its crude fiber content and feed value to roughage at a later stage of ripening (see *Table 2*). The guaranteed tannin content (condensed tannins) corresponds to the reference test of VÁZQUEZ-CARILLO et al. (2020) (60.7 g/kg DM). The chip length was specified as 1 cm; testing several samples actually showed a

Figure 5: Creation of total mixing ration

Figure 6: Grass silage, a typical component in cattle feeding in Austria

length of 0.9 cm. Lemongrass is a very high-yielding crop of the tropical and subtropical regions of the world. Lemongrass does not tolerate frost, and is grown there on arable land. The plantations can be used for up to 8 years. With four harvests per year, up to 20 tons of DM/ha of high-quality lemongrass can be produced. The lemongrass, which is currently imported into Europe in small quantities, is used for the extraction of the active ingredients or as a seasoning and luxury food. In order to make economic use of cattle fattening, prices still have to fall by a factor of 10. This is conceivable in an organized production and trade chain.

Table 2: Ingredients and energy of lemongrass

Feed	Weender nutrients and fiber components											Energy		Condensed Tannins
	FM	XP	XL	XF	XA	XX	OM	NDF	ADF	ADL	NFC	ME	NEL	
	g/kg DM											MJ/kg DM		g/kg DM
Lemongrass	912	75	30	328	75	492	925	654	347	52	166	8.59	4.96	>60

Table 3: Trial setup

	Feeding 100 g of lemongrass per animal per day			
	1. Repetition		2. Repetition	
	Mesurement period			
	1	2	3	4
Box	21.09-09.10	10.10-30.10	31.10-20.11	21.11-11.12
①	with	without	with	without
②	without	with	without	with
③	with	without	with	without
④	without	with	without	with
⑤	with	without	with	without
⑥	without	with	without	with
⑦	with	without	with	without
⑧	without	with	without	with
Order	①→⑧	①→⑧	⑧→①	⑧→①

With constant ad libitum feeding of the ration from *Table 1*, individual stalls were administered a dose of 100 grams of lemongrass per animal per day between 21.09.2020 and 11.12.2020 according to the schedule in *Table 3*. Two adjacent stalls formed a group, which was fed alternately once with and once without lemongrass. With four measurement periods, there are two complete repetitions with an interval of six weeks. Because the measurement time (described below in more detail) is about 6 hours for a complete measurement of all animals, and during this time the natural cycle of digestion also progresses, the measurement sequence between the repetitions was reversed. This means that in the first repetition was measured from the youngest to the oldest animals, and in the second repetition from the oldest to the youngest. This measure ensures that a possible influence of the measurement duration of the individual animals can be balanced out in the overall trial.

Measurement

CH_4 measurement of breath

For legal reasons, only very short measurement intervals are possible for each individual animal in the AREC‘s respiratory chamber. An attempt with repetitions cannot therefore be carried out. This means that an alternative technique had to be used to measure CH_4 emissions. Based on the findings of SORG et al. (2017, 2018), the portable measuring device Laser *methane mini SA 3C32A-BE* (LMm) from Tokyo Gas Engineering Solution was selected. This device is the successor to the laser methane detector (LMD) measuring device from the same company with which a series of reference measurements for other CH_4 measuring systems were carried out. The measurements under laboratory conditions have shown high correlations to closed systems (respiration chamber).

The measurement with the LMm is based on the excitation of CH_4 molecules by a high-energy, green laser of class 3R and its reflection (albedo) from surfaces. The chance of encountering CH_4 molecules depends on the length of the pulse, which is why the unit of measurement is "ppm m". One ppm (part per million) is equivalent to one gram per ton. The m expresses the running length of the signal. The LMm delivers two readings per second in active use. This high density of measurements means that, with the selected measurement period of 5 minutes per animal, a whole spectrum of measurement values is available at the end. This spectrum provides information about the CH_4 concentration in the breath. The absolute load cannot be determined, but the concentration value in the breath has a high correlation to the load at the same measurement time.

Practical use shows that the real challenge of field measurement is not measurement technology for determining the CH_4 concentration in the measurement atmosphere, but its consistency. In the run-up to the trial, test measurements were carried out over several weeks on individual animals under constantly changing environmental conditions. These activities show that the measured value as an absolute variable fluctuates far beyond the variance of the effects. The differences between the same animals were largely retained. The mobile measurement with the LMm should therefore not be used to determine absolute values. Measurements that are made "ceteris paribus" may, however, analyze the difference between the groups studied. The following steps further the success of the measurement of a test group with the LMm:

- Low background levels in the atmosphere: If a barn has to be closed for weather-related reasons (cold snap, strong wind, etc.), the concentration of all gaseous emissions increases rapidly. This enriched atmosphere changes the measurement spectrum in that higher values are measured in absolute terms, and the sensitivity of the measuring ranges at the edge of the spectrum decreases. Here, they measure lower minima or maxima relative to the middle of the spectrum. A high background level attenuates the breadth of the spectrum, which is why the measurements should be taken when the barn can be well ventilated under windless conditions. Nice spring and autumn days offer good basic conditions for this.
- Calm measuring conditions: CH_4 has about half the density of the ambient air and will therefore always escape upwards relatively quickly. This quality is further negatively affected by additional air movements. Such movements can also cause the CH_4 breath cloud, which forms in the area of the cattle heads, to swirl rapidly with the surrounding air. For the measurement, therefore, all animals should be restricted to the immediate area of the group and no other movements should take place in the measuring range. Ideally, an existing forced ventilation is switched off, interference with external air access is prevented, and the entire measuring cycle is

held in the system at the best possible stillness. The measuring technician should be alone in the stable and only move slowly. Each group must be given the chance to build a CH_4 breath cloud, which entails a minimum waiting time of 5 minutes before the measurement.

- Compact, fixed group: Even if we measure individual animals, in the end we are only interested in the effect of the whole group. Of course, individual animals differ in their individual emissions, but these cannot be measured precisely with the LMm, because adjacent animals in the CH_4 breath cloud influence each other. It is therefore important to measure animals either individually or, as in this case, in compact groups. At the trial farm, all animals in a group can be kept at a short distance from the feeding. There is, therefore, an accumulative effect among the animals. This is statistically confirmed in the trial.

The measurement

For the measurement, the technician sits opposite the cow at a distance of 1.7 meters in such a way that the mouth of the cattle is at the same height as the LMm and the angle between the mouth and the laser beam is 90°. The technician needs a suitable seat for the long measuring time of 5 minutes in which he holds the measuring instrument with his hands out in front, following the mouth of the animal constantly in a slow rotating motion. It is of the utmost importance that the technician is aware of the effect of the laser in class 3R! The laser radiation is in a potentially dangerous wavelength range for the eye, which is why the head of the animal is deliberately separated into a measuring area and a safety area. A fixed assembly of the device could cause the light to enter the eye of the animal, albeit for a short period of time. Manual operation is the safest way to ensure maximum protection of the animal.

If the measurement technician goes to work with the necessary calmness, the beef cattle show little fear of the unfamiliar situation. The light source of the green laser is visible on the device, but not the measuring point at the muzzle itself. This is where a ruminant‘s eye arrangement is helpful. Since the eyes are on the side of the skull, the focus of the gaze is not particularly high.

Figure 7: Appearance and description of the LMm

Gas	methane (CH_4) und Methangase mixtures (natural gas or similar)
Measuring range	1 to 50.000 ppm m
Accuracy	± 10 % in the measuring range
	between 100 and 1,000 ppm m
Measuring speed	0,1 Seconds
Measuring distance	0.5 bis 30 meters
Environment conditions	-17 to +50° Celsius
Laser	Class 3R

Company contact: Pergam-Suisse AG, Birmensdorferstr. 125, 8003 Zürich, Schweiz
email: info@pergam-suisse.ch | www.pergam-suisse.ch

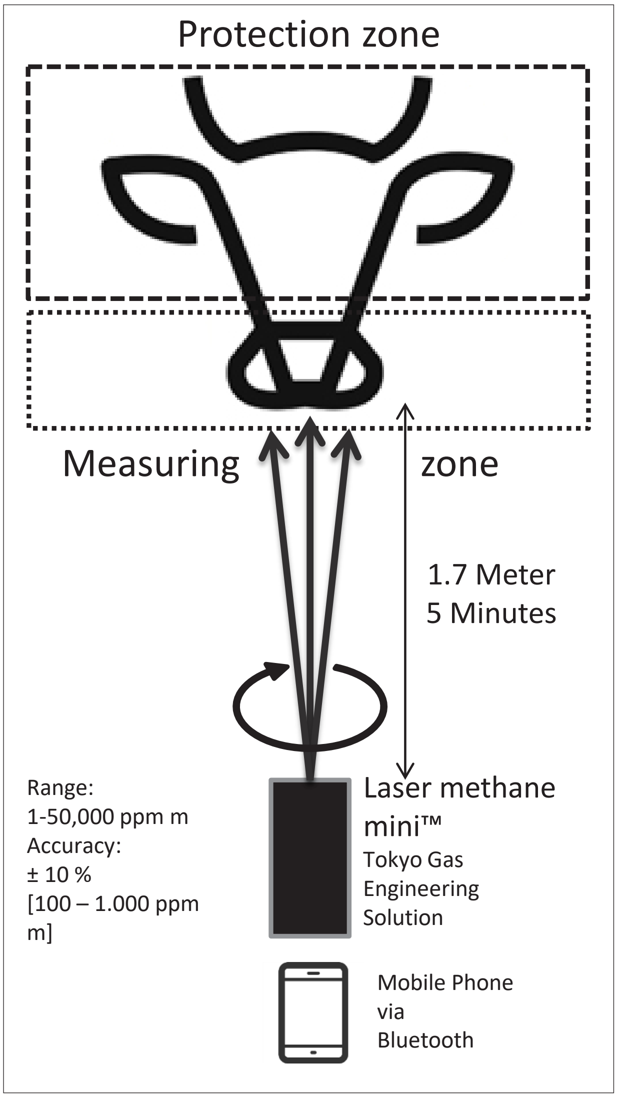


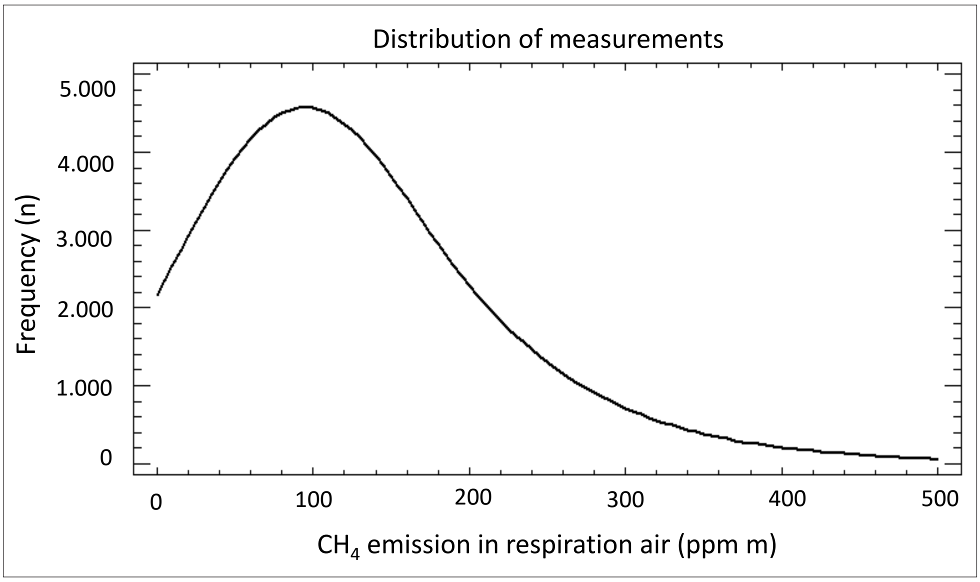
Figure 8: Measuring arrangement

Preparation of CH_4 measurements

For each measurement, the LMm delivers around 600 individual values (5 minutes x 60 seconds x 2 values per second) that cover a wide measuring range. The reasons for the variability are the natural breathing cycle of the animals, the emission dynamics of the rumen, the measuring atmosphere, the measurement and the test question. *Figure 9* the right-skewed distribution of the more than 100,000 individual measurements. The skewness and curvature deviate widely from the normal distribution, which is why an angle transformation must be carried out for the group comparison of the entire data set. In order to investigate the local effect of the test question within the variability of the measurement, the data set of each animal was converted into relevant quantities of descriptive statistics. Ultimately, the following areas of the measurement signal can be examined:

- four quartiles to check the relationship along the ascending values
- two 5% width margins to test Minima and Maxima
- an average to evaluate the overall relationship

Figure 9: Distribution of all individual measurements



Dynamic measurements in the rumen

The colonization of the rumen with bacteria and protozoa is a natural process that is closely linked to the food supply. Of the different species that specialize in different feed components, the necessary species are always available. Their frequency correlates to a high degree with the job they are given through feeding. If we intervene in this natural structure with feed additives, we must be aware that we can also interfere with the natural processes.

The possible influence of the addition of lemongrass on the conditions in the rumen is investigated in the field test by the use of measuring boluses from smaXtec. These boluses are inserted directly down the throat via the mouth with a guide tool. From there, the boluses slide into the rumen, where they remain in the reticulate stomach because of their weight. The following measuring boluses were used in the investigation:

The data of the smatec-boluses are continuously transmitted via a radio connection to the data service of the company and can be read from there in a processed form via a dashboard. This access to your own data is a real service for scientific experiments, because possible effects can be tested in real time and the harvesting of the data is very easy. In the trial, 16 measuring boluses were used. One animal in each stall was equipped with the sensor „Classic" SX.2, another with the sensor „pH Plus". The animals were selected randomly. The entry of the boluses was made somewhat late in the current first measurement period, because a new generation of technology was used for this study. Scientists around the world recommend this technology for ruminant testing. It is uncomplicated, cheap, and provides a high density of metrics for your own statistical assessments.


Figure 10: Boluses from the company smaXtec

smaXtec Classic Bolus SX.2

Sensors:
- Temperature
- Kinematic acceleration sensor
- Wireless sensor for data transmission

Measuring profile:
- Total measurement time: up to 4 years
- Time resolution: 10 minutes
- Internal reading memory: 6 days
- Relative measurement accuracy of temperature measurement: ± 0.05°C

Derived management information for dairy cows:
- Early detection of diseases
- Heat detection
- Calving alarm
- Monitoring of feeding (measurement of chewing activity, control of water consumption)
- Alarm in case of heat stress

smaXtec pH Plus Bolus

Similar to smaXtec Classic Bolus SX.2 but with the following additions:

+ Sensors:
- pH-Sensor

Measuring profile:
- Total measurement time of the pH sensor: 150 dayse
- Internal reading memory: 50 days
- Accuracy of pH measurement: up to the 90th measurement day ± 0.02 after that 0.04

Additional management information for dairy cows:
- Evaluation of feeding with a view to possible subacute rumen acidosis (SARA)

Company contact: smaXtec | Belgiergasse 3 | 8020 Graz, Österreich
info@smaXtec.com | https://smaxtec.com

In vitro test of gas formation

In parallel to the field trial, a small amount of lemongrass was finely ground and tested for its gas formation using the *in vitro* method, Hohenheim Feed Value Test (HFT) (*figure 11*). The study was carried out in one run consisting of 54 measuring flasks. Flasks 1 to 6 showed the unaffected reference gas formation of the rumen juice. In the remaining samples, a sliding transition between the hay standard of the HFT and the lemongrass was investigated. The following levels of exchange were examined: 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%.

Statistical evaluation

The program Statgraphics Centurion XV was used to evaluate the relationship between the classes and the dependent parameters. A general linear model (GLM), Type III, which was used in several configurations, was used for the group comparison. An analysis of variance, a residual analysis and Least Significant

Figure 11: Hohenheim Feed Value Test

Difference (LSD) were used within the GLM. The check for normal distribution of the CH_4 values showed a right-skewed data position, which was corrected by an angle transformation. The smaXtec data is normally distributed.

Evaluation of CH_4concentration in breath with the LMm

Y_{ij} = $\mu + Z_i + MP_j + Z_i \times MP_j + \varepsilon_{ij}$

where

y_{ij} = Observation value of the dependent variable → Minima, Maxima, Medium, 1st Quartile, 2nd Quartile, 3rd Quartile, 4th Quartile

μ = common (middle) constant

Z_i = fixed effect of feeding (with/without lemongrass)

MP_j = fixed effect of the measurement period (1 to 4)

$Z_i \times MP_j$ = Interaction between feeding and measuring period

ε_{ij} = Unexplained rest of the spread

Evaluation of data from smaXtec boluses

$$Y_{ijk} = \mu + Z_i + MP_j + S_k + Z_i \times S_k + \varepsilon_{ijk}$$

where

y_{ijk} =	Observation value of the dependent variable → pH-value in the rumen, temperature in the rumen without drinking phase
μ =	common (middle) constant
Z_i =	fixed effect of feeding (with/without lemongrass)
MP_j=	fixed effect of the measurement period (1 to 4)
S_k=	fixed effect of the hour (1 to 24)
$Z_i \times S_k$ =	Interaction of feeding and hour
ε_{ijk} =	Unexplained rest of the spread

Results

Ration and nutrient supply of beef cattle

Feed intake, energy and protein concentration

Figure 12 shows the polynomial course of the calculated feed intake depending on the age for a fattening process with an average daily increase of 1,350 grams. The left figure also contains an indication of the age of the individual groups at the beginning of the trial. Together, they span a period of 158 days. At the beginning of the trial, the youngest group had a calculated weight of around 293 kg, the oldest group at the end of the trial 607 kg. Therefore, the field test covered the second half of the usual Austrian bull fattening well. Feed intake increased steadily during this period and we can assume that the youngest group at the start of the trial took an average of 5.5 - 6.0 kg of dry matter and the oldest group at the end of the trial took an estimated 8.5 - 9.0 kg dry matter. Although this assessment is not fundamentally necessary to interpret the measurement results of the sensors, it becomes clear that with increasing live weight, the constant amount of 100 g of lemongrass was an ever smaller proportion in the ration.

The optimal nutrient concentration of energy and protein for good growth is shown in the right part of *Figure 12*. Between the 250th and 400th day of life, the demand for nutrient concentration in the feed decreases. Cattle over 300 kg live weight already have such a large feed intake capacity that the sum of the nutrient requirements increasingly depends less and less on the concentration. The energy concentration of 10.8 MJ ME/kg DM determined in *Table 1*, and the protein concentration of 139 g/kg DM, are constant in the dynamic course due to single-phase feeding and cover the nutrient requirements from the 250th day of life. The fattening cattle studied are therefore not limited in their free nutrient supply.

Calculated proportion of lemongrass in the ration

A constant quantity of 100 g of lemongrass is diluted in the course of the study, as shown in *Table 4* due to the ever-increasing feed intake of the individual groups. If 100 g of lemongrass at the beginning of the trial in the youngest group represents 1.70 % in the ration, this value will fall to 1.14 % by the last measurement period. The quantity used is compared to the work of VÁZQUEZ-CARILLO, 2020 between the trial 1 and the group 2% in trial 2.

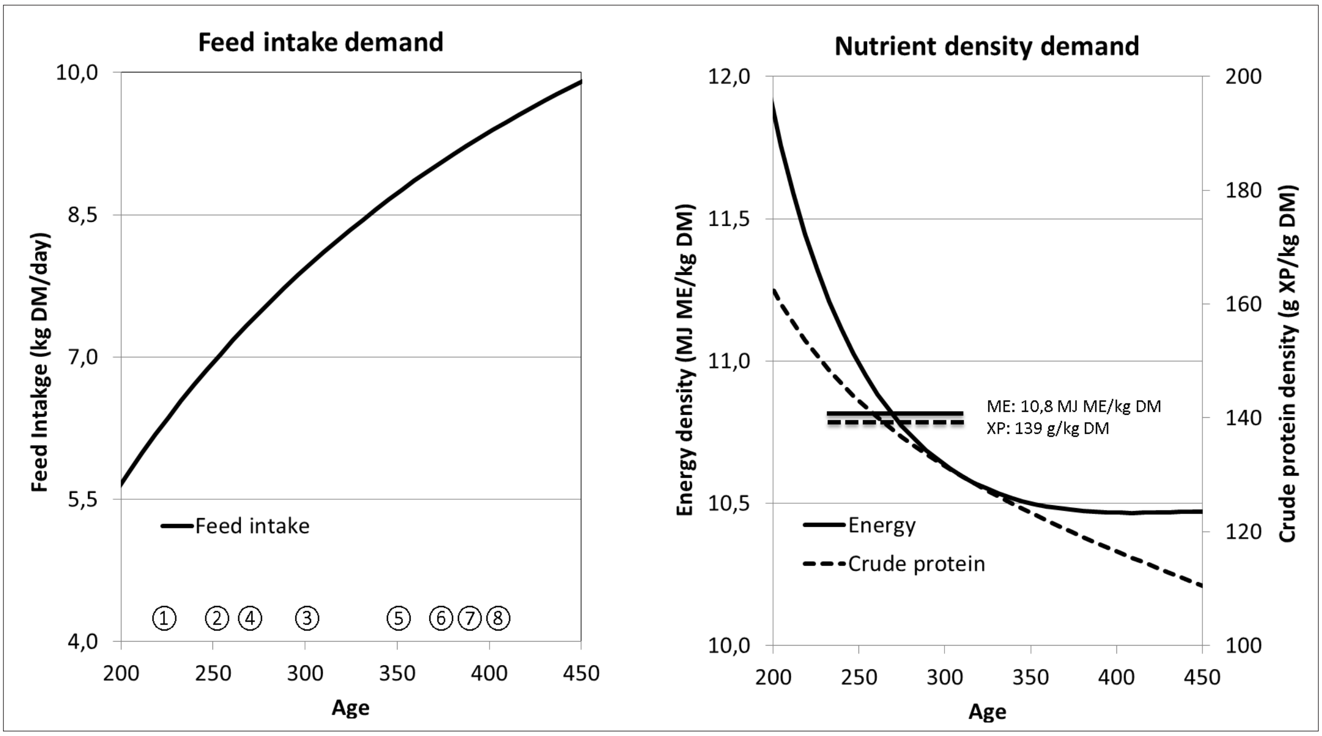
Results of CH_4 concentration in the breath

The application of the described statistical model with the two classes of feeding and measuring period leads to the results in *Table 5*.

The following interpretation can be made for the two classes:

- Feeding lemongrass: Along the ascending parameters of the descriptive description, Minima and Maxima of the group with lemongrass span a range of 56.2 to 299.8 ppm m. In the group without lemongrass, a higher level is reached with the range of 69.1 to 322.5 ppm m for all parameters. The absolute difference between the two feeding groups slowly increases along the spectrum from 13.0 to 22.7 ppm m, with the feeding of lemongrass always leading to lower values. The two groups differ highly over 95% of the measurement profile. Only the difference between the maxima can no longer be verified statistically. On average, the addition of 100 grams of lemongrass

Figure 12: Feed intake and necessary nutrient concentration of fattening cattle



per animal per day resulted in a highly significant reduction of CH_4 emissions in the breath of 14.6% (*Figure 13*).

- Influence of the measurement period: The conditions required for the description of the measuring period could not be consistently adhered to in the field test. A bad weather period with strong northerly winds in the last week of October and the lower daytime temperatures in December meant that the roll curtains were not always equally wide open and therefore the overall concentration in the fattening barn differed between the measuring periods. In particular, the situation in the second measurement period meant that these differed significantly overall. This difference also extends to the repetition described in the experimental design. The variation resulting from the different measurement periods is a multiple of the influence of the feeding question. This realization confirms that it is absolutely necessary to carry out all measurements within a measurement session in the shortest possible time.

Table 4: Calculated proportion of lemongrass in the total ration

	Calculated proportion of lemongrass (%)[1] **in total ration**			
	Measuring period			
	1	**2**	**3**	**4**
Box	**21.09-09.10**	**10.10-30.10**	**31.10-20.11**	**21.11-11.12**
①	1.70	-	1.50	-
②	-	1.47	-	1.35
③	1.39		1.29	
④	-	1.43	-	1.31
⑤	1.30	-	1.22	-
⑥	-	1.21	-	1.16
⑦	1.24	-	1.18	-
⑧	-	1.19	-	1.14

[1] with a constant administration of 100 grams of lemongrass per animal per day

Table 5: Results of statistical analysis

Parameter		**Lemongrass (Z_i)**		**Measuring period** (MP_j)				**MAE**	**p-value**			**R^2**
		with	**without**	**1**	**2**	**3**	**4**		**Z_i**	**MP_j**	**Z_i x MP_j**	
Methane concentration in the breath (CH_4)												
Minima	ppm m	56.2	69.1	42.3	92.0	36.6	79.6	21.9	0.005	0.000	0.503	43.7
1. Quartile	ppm m	72.0	88.6	58.1	116.5	49.7	96.9	25.6	0.003	0.000	0.511	43.3
2. Quartile	ppm m	89.4	109.0	76.1	143.9	62.8	114.1	29.1	0.002	0.000	0.575	43.7
3. Quartile	ppm m	113.0	135.6	99.5	180.1	80.1	137.5	33,3	0.003	0.000	0.678	44.3
4. Quartile	ppm m	162.2	185.8	146.6	245.8	115.5	188.0	42.1	0.013	0.000	0.831	43.7
Maxima	ppm m	299.8	322.5	276.5	400.3	223.4	344.6	6.9	0.116	0.000	0.653	37.0
Mean	ppm m	109.1	129.7	95.1	171.6	77.0	134.1	32.0	0.004	0.000	0.691	44.2

Figure 13: Results of feeding 100 grams of lemongrass per animal per day

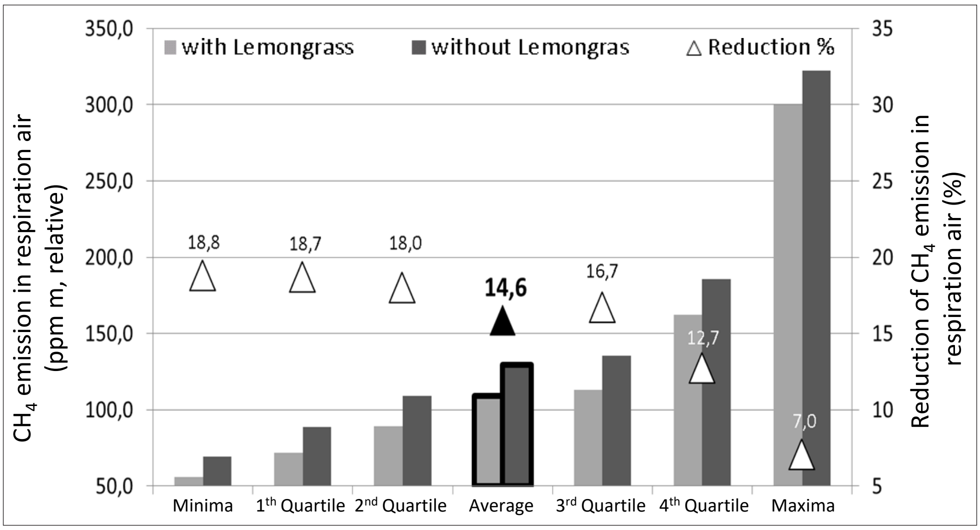
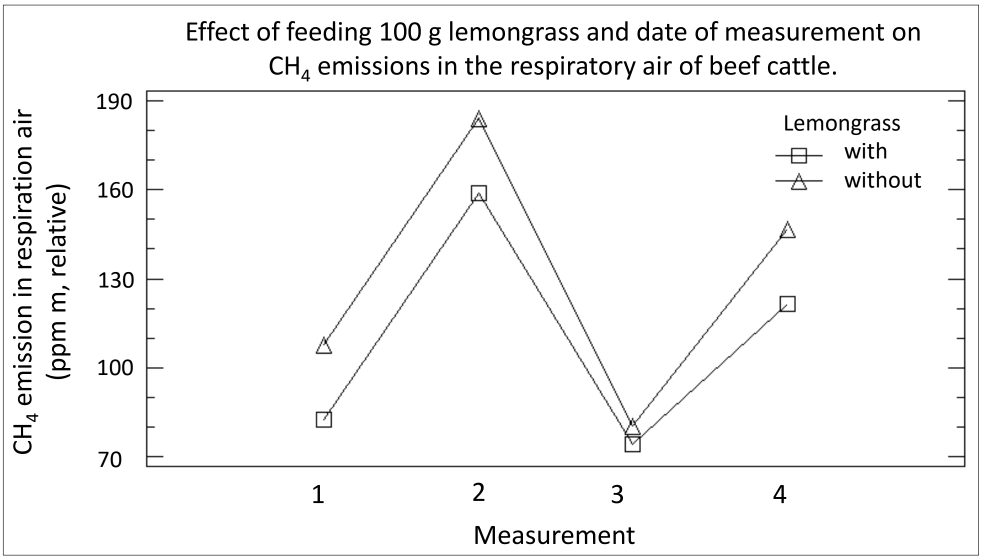


Figure 14: Interaction of the test question with the influence of the measurement period



- Interaction between the feeding groups and the measurement period: The interaction of the two classes is not significant. This means that the results of the feeding group shift within the measurement periods, but always remain approximately the same in themselves. Approximate because, as shown in *Figure 13*, the distance between the feeding groups is not the same for each measurement period. The smallest distance of 7.8% was achieved in measurement period 3. In measurement period 1, the maximum distance of 23.4% was achieved.

Results of measurements with smaXtec sensors

The application of the described statistical model with the three classes feeding, measuring period and hour leads to the results in *Table 6*.

The following interpretation can be made:

- Feeding of lemongrass and measuring period: The addition of 100 grams of lemongrass did not make any difference in most parameters compared to the group without addition. The individual measurement periods (period 1

Table 6: Results of statistical analysis

Parameter		**Lemongrass** (Z_i)		**Measuring period** (MP_j)				**Hour** (Sk)	**MAE**	**p-value**				R^2
		with	**without**	**1**	**2**	**3**	**4**	**0-23**		Z_j	MP_j	S_k	Z_{jj} x S_k	
Analyses in the Rumen (smaXtec-Sensor)														
pH		6.47	6.46	-	6.45	6.47	6.49	Figure 15	0.028	0.124	0.000	0.000	1.000	86.4
Temperature	°C	38.93	38.91	-	38.90	38.96	38.90	Figure 16	0.047	0.247	0.000	0.000	0.006	80.1
Drink	n	10.8	9.9	-	-	-	-	Figure 18	2.39	0.225	-	0.000	0.760	74.9
Aktivity	[0,100]	7.7	7.1	-	7.7	8.0	7.5	Figure 17	0.76	0.002	0.009	0.000	1.000	78.7

was not used due to the lack of time completeness) show a significant difference, but this is marginal. The high degree of explanation of the models (the coefficient of determination R^2 is always over 70%) is probably due to the effect of the temporal dynamics in feeding and digestion.

- Temporal dynamics of the measured values over the course of the day (*Figure 15, Figure 16, Figure 17* and *Figure 18*): The daily fluctuations in the pH in the rumen are an expression of the feeding and digestive dynamics of ruminants. In the period from midnight to about 6:00 in the morning, the trial animals rest. They do not eat during this time, they hardly absorb water, and have a low activity value. Therefore, the temperature in the rumen remains constant during this period, but the pH-value rises slowly, because the supplied saliva buffers the acids. The acids formed, which are an important part of the energy metabolism of cattle, are also absorbed. In the morning and evening hours, driven by the feeding cycles and the feed template, two peaks result in the parameter activity and water absorption. These can also be observed indirectly at the pH value in the rumen. This drops with the first peak after 6:00 by about pH 0.12, then forms a plateau over the day before falling again after the second activity phase by pH 0.12. The temperature in the rumen does not follow the feeding cycle, but the body temperature of the cattle. The graphs in the figures below show that the differences between the feeding groups were in fact sometimes only marginal. The figures show the described dynamics greatly increased because the areas of the y-axis are very sharply dissolved.

Results of the in vitro approach test

The *in vitro* probe shows that the gas formation decreased significantly at the addition of 1% lemongrass (GB_8 = 17.9 ml, GB_{24} = 35.7) based on the gas formation of the hay standard (GB_8 = 21.4 ml, GB_{24} = 42.4 ml). The decrease was 15.8%. After that, the formation of gas continued to decline, with the dynamics of the two measuring times differing. The measurement after 8 hours gradually flattens out. The 24-hour measurement first forms a plateau and then drops more strongly. *Figure 19* clearly shows this development. When pure lemongrass was used in the study, GB_8 gave a value of 4.9 ml and GB_{24} a value of 16.7 ml. The resulting energy content was 4.9 MJ ME/kg DM. This is dramatically less than the energy determination with the ELOS method, which yielded an energy

Figure 15: pH-Value in the rumen

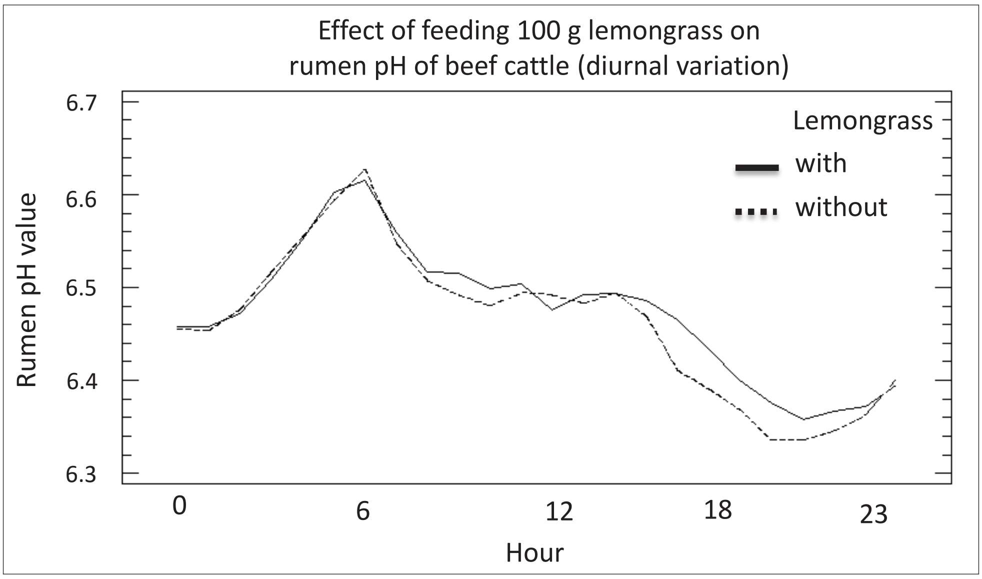


Figure 16: Temperature in the rumen without drinking

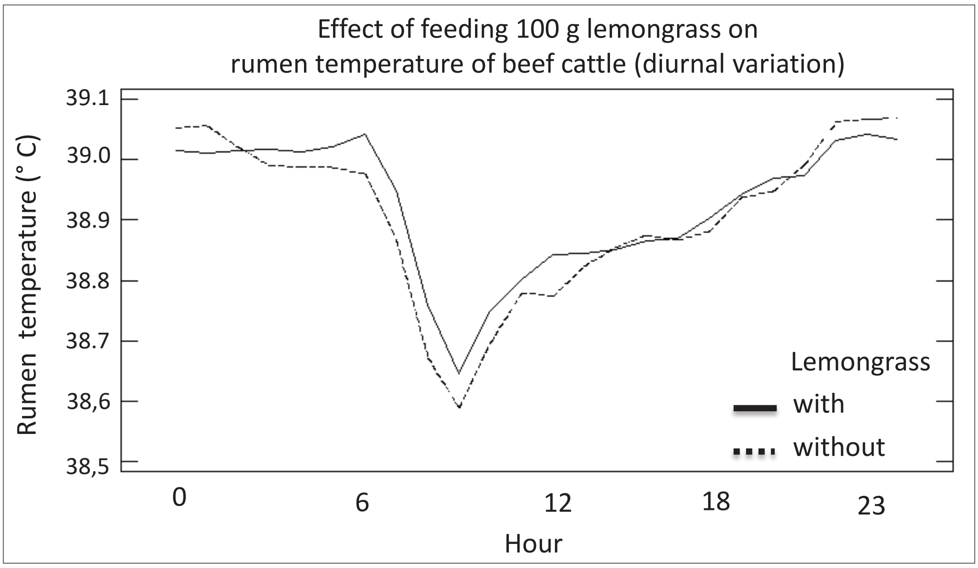
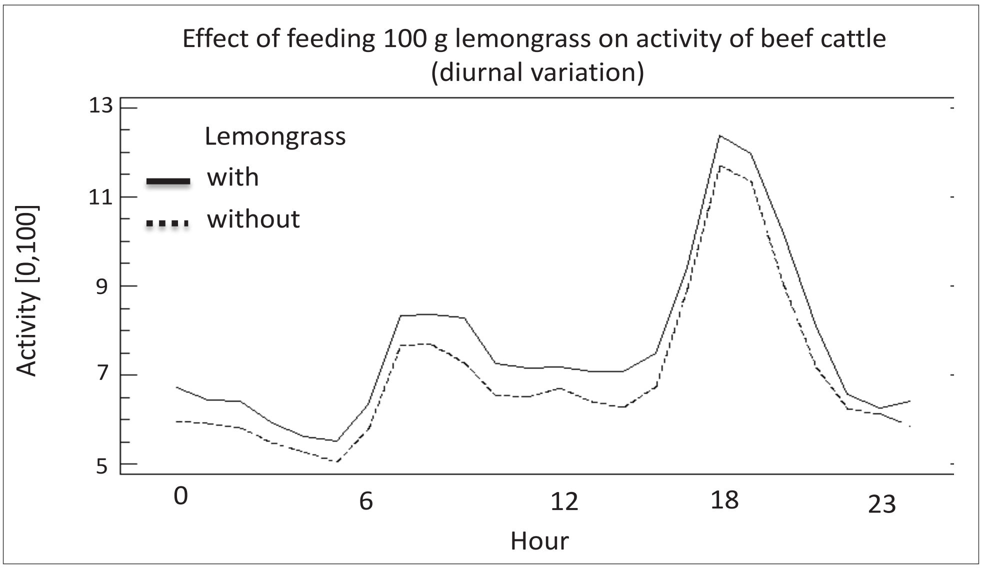


Figure 17: Activity of trial animals [0.100]



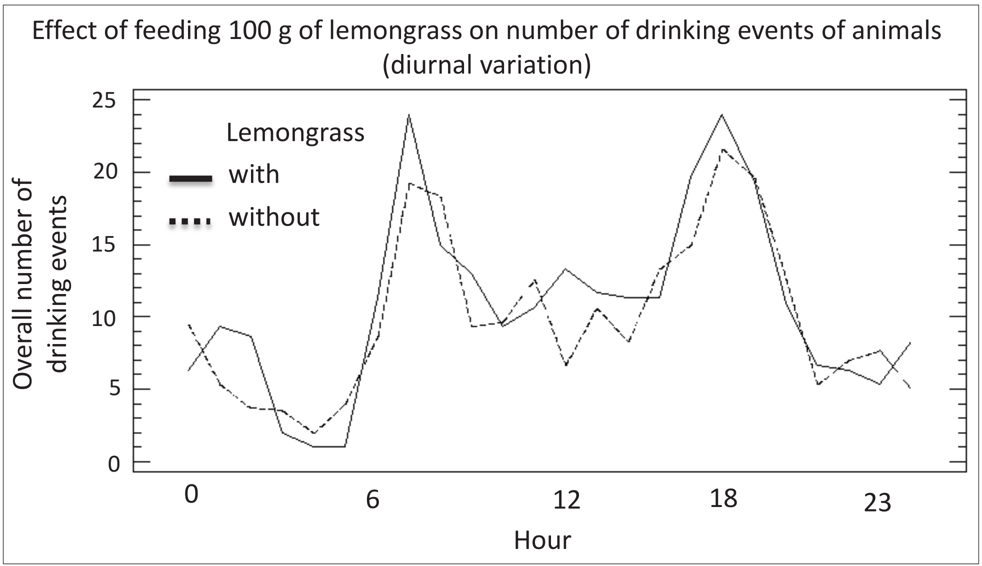


Figure 18: Total water intake

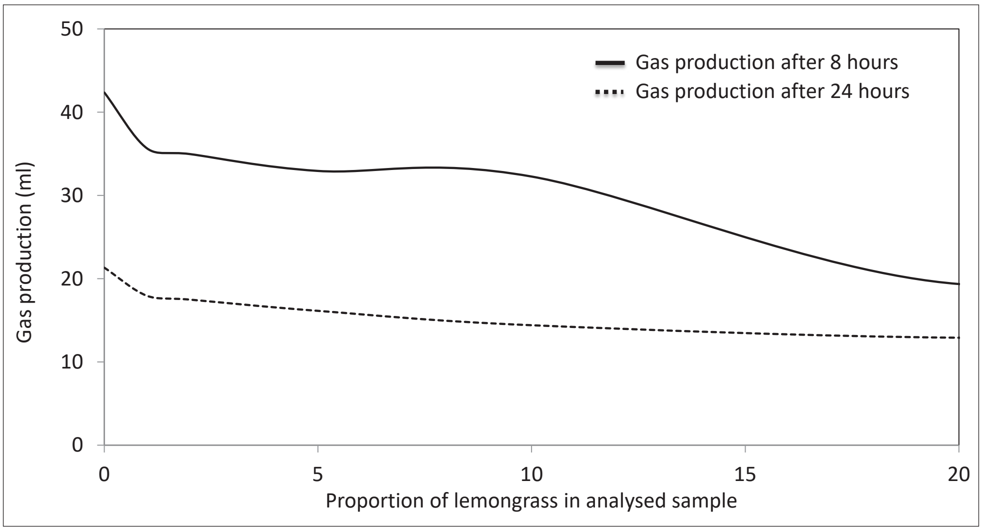


Figure 19: Influence of lemongrass on *in vitro* gas formation

content of 8.59 MJ ME/kg DM (*Table 2*). This result, even if carried out only as a touch test, confirms the statements on the effect of large amounts of tannin on the microbes in the ruminant.

Summary and discussion

Methane (CH_4) acts as a greenhouse gas (GHG). One of the emission pathways leads deep into the interior of ruminants that have entered into a symbiosis with bacteria and protozoa for the digestion of structurally rich food. This relationship allows cattle to produce milk and add live weight from hard-to-digest complex carbohydrates such as those available from grassland or silage maize cultivation. CH_4 is released as a by-product of the break-up of these carbohydrates.

As part of its climate protection legislation, the international community is calling for a reduction in total GHG emissions and is also demanding its contribution from agriculture. This can only be achieved by two paths in terms of reducing CH_4 emissions from ruminants. The first, more organizational and far more powerful path is to reduce the animal population to a sustainable level. The second, the technological path, is to influence enterogenic fermentation in ruminants. Currently, a number of natural and chemical substances are known that can have a dampening effect. One of these substances is tannin, which is present in lemongrass with a 6% content.

Based on a study by the Autonomous University of the State of Mexico (VÁZQUEZ-CARILLO et al. 2020), the globally operating company Burger King currently recommends an addition of 100 grams of lemongrass per animal per day to cattle fattening and claims as a result a reduction in CH_4 load between 15.3 and 26.3%. The AREC Raumberg-Gumpenstein (8952 Irdning-Donnersbachtal) carried out a field trial in cooperation with the company Marcher Fleischwerke (9524 Villach) and the farmer Christian Schrammel (2625 Schwarzau am Steinfeld) to verify this recommendation under Austrian conditions. For the field test, 47 fattening bulls in the weight range between 300 and 600 kg were selected in 8 stalls of 6(5) animals each. These animals were fed alternately with or without the addition of lemongrass in four measuring periods of three weeks each with the same ration. At the end of each measurement period, the CH_4 content in the breath of each animal was measured with the device Laser Methane mini (LMm) from Tokyo Gas Engineering Solution. The absolute results of these measurements depend heavily on the environmental conditions in the barn and the technique used. This article therefore also describes the measurement process very precisely. Two animals per group were additionally given a measuring bolus from the company smaXtec via the mouth into the rumen. These measuring sensors continuously provide information about the pH value and the temperature in the rumen. Additional kinematic measurements can be used for activity control. All feed was chemically analyzed, and a dynamic series of measurements with the Hohenheim feed value test was carried out for the lemongrass. In this test, the gas formation was checked at different proportions of lemongrass in the sample. All planned measurement data were successfully collected and statistically evaluated in the period between September and December 2020.

The results of each single measurement of CH_4 with the LMm, which show the CH_4 concentration in the breath, were tested not only as a mean value, but at the 5% wide margins and the four quartiles. It was found that an addition of 100 grams of lemongrass per animal per day reduced the CH_4 concentration in the breath over the entire spectrum. The effect on the quartiles ranged from 18.7% in the first quartile to 12.7% in the fourth quartile. On average, the addition of 100 grams of lemongrass reduced the CH_4 concentration in the breath by 14.6%. However, the result may differ significantly from this value for individual measurements. The highest reduction was achieved with 23.4% for the first measurement and the lowest with 7.8% for the third measurement. The *in vitro* study also showed a 15.8% reduction in gas formation with an addition of 1% lemongrass.

Both the reduction in the CH_4 concentration in the breath of the animals (14.6%, 7.8% – 23.4%) and the decrease in gas formation in vitro trials (15.8%) are in the reference range (16.4% - 26.0%) of the reduction of CH_4 loads at VÁZQUEZ-CARILLO et al. (2020). As in previous studies with tannin-containing feed additives (e.g., STAERFL et al. 2012, BEAUCHEMIN et al. 2020 and NIDERKORN et al. 2020), a methane-reducing effect was also observed in the use of lemongrass. This is probably due to the high content of condensed tannins in the lemongrass and the resulting reduced ruminant degradability of the fiber components (GOEL and MAKKAR 2012). Since methane is produced in the rumen over the course of the degradation of fiber components, this results in the methane-reducing effect of lemongrass.

The desired effect can be clearly identified and a wide use of feed with a high tannin content can be recommended when: a.) the reduction effect is consistently confirmed over an entire fattening period and b.) the overall reduction in digestibility of fiber-rich rations remains low. The cost of a place in cattle fattening is 36 kg of lemongrass per year. These costs must be covered either by market forces or public subsidies.

The statistical evaluation of the measurements of the smaXtec measuring boluses showed only marginal differences with regard to the addition of 100 grams of lemongrass per animal per day. It can be assumed that the addition of this small quantity does not reduce the digestibility of the fattening ration with an energy content of 10.8 MJ ME/kg DM, a crude protein content of 139 g/kg DM and a crude fiber content of 218 g/kg DM. The central parameter of the daily fluctuations of pH and temperature in the rumen is the time in the constant dynamics of eating, chewing and digestion. The class hours of statistical evaluation reflects the course of this dynamic as well as the activity of the animals and the water intake. The smaXtec rumen sensor is a really good tool for observing the processes in the rumen and is recommended for use by practitioners and other research groups.

Research to be done

- If feed additives such as lemongrass are to be used in the future to reduce the CH_4 loads in agriculture, exact tests will have to be carried out. Uncertainties exist here mainly in the assessment of the entire production cycle, and possible negative effects on the production output of animals, which cannot be observed here.
- The field of research in microbiology must be intensively developed in order to better understand the complex interrelations in the rumen. Only with this knowledge can targeted steps be taken to reduce CH_4 emissions in agriculture.
- The question of CH_4 emissions must not end in the evaluation of concentrations per product unit, but must be understood as total load (including all greenhouse gases). In addition, the assessment of production systems must be extended to all environmental impacts.

Thanks

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Lemongrass

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