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Integrating Activities for Starting Communities

SmartCow: an integrated infrastructure for increased research capability and innovation in the European cattle sector

SmartCow
an integrated infrastructure for increased research capability and innovation in the European cattle sector

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SmartCow
an integrated infrastructure for increased research capability and innovation in the European cattle sector

EXECUTIVE SUMMARY

Background	In the frame of SmartCow project, IRTA has made access it Research Installation "EVAM" through Trans National Access (TNA).
Objectives	This Deliverable aims at describing the TNA provided by IRTA during the SmartCow project.
Methods	The Deliverable is composed of a table summarising the TNA provided by the Research Installation (RI) and by the reports of activities provided by the TNA users who accessed this RI.

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1 TNA provided

Name of the TNA project	Name of TNA user	Organisation of TNA user	Country of TNA user	Installation from the RI	Start date	End date	Number of units of access provided
TNA1. Evaluation of zinc oxide source and dose	Denise Cardoso	ANIMINE	France	EVAM	01/03/20	31/05/21	720
TNA 2. Inclusion of seaweed on milk production, feed efficiency and rumen microbiome of dairy cattle	Katerina Theodoridou	University of Belfast	Ireland	EVAM	01/08/20	31/03/22	480
TNA 3. Strengthen Laser Methane m-g device measurement protocol to estimate methane emission of dairy cow ruminants directly in any commercial farm.	Boré Raphaël	IDELE	France	EVAM	01/10/20	30/03/21	100
TNA 4. Effects of live yeast supplementation on dairy cows	Tanja Kotevska	GreenAgro	Macedonia	EVAM	01/04/21	30/04/22	288

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2 Final reports of the each TNA provided

2.1 TNA 1

Objective

HiZox® (Animine, France) is a potentiated zinc oxide for animal nutrition showing a superior bioavailability compared to standard sources of zinc (Cardoso, 2021). The objective of this study was to evaluate the supplementation effect of HiZox® fed below the maximum level of zinc authorised in the EU (120 ppm zinc) (EU Reg 2016/1095), on performance, milk quality, serum Zn concentrations and health status in lactating dairy cows.

Material and Methods

Sixty multiparous and primiparous Holstein cows were split in 3 pens with 20 cows in each equipped with 20 cubicles, 15 bin-scales to offer the TMR, and 4 water throughs.

Each group received the following zinc supplementation during 84 days (12 weeks, from September to December 2020):

Table 1. Experimental diets

	Zn in the basal diet (mg/kg DM)	Zn from potentiated ZnO (HiZox®) (mg/kg DM)	Total Zn (mg/kg DM)
T40	40	0	**40**
T60	40	20	**60**
T90	40	50	**90**
T120	40	80	**120 (Max EU Zn level)**

All cows were fed the same TMR supplemented with a common premix of vitamins and minerals with a Zn dose to achieve daily requirements (40 mg/kg DM) considering 25.5 kg DM intake

Cows were fed twice daily the common TMR in the bin-scales after the morning (08:00 am) and afternoon (07:00 pm) milking. Extra Zn supplementation was offered mixed with pelleted soybean in the milking parlour according to the treatments and their TMR intake.

The following parameters were recorded:

- Daily: individual feed intake, milk yield and composition, body weight, feed efficiency
- Initial/final: serum Zn concentration (5 cows/treatment)
- Final: milk fatty acid profile (10 cows in T40 and T120)
- Quarters with mastitis during the study

Statistical analysis

Data from the last week (week=12) of experiment (except for serum Zn, week=11) were analyzed using proc GLM, SAS software to investigate the linear and quadratic responses of incremental levels on Zn in the experimental diets. Intake and performance data were analyzed in proc MIXED, SAS software and differences between all treatments were controlled for multiple comparisons using the Tukey procedure. Since data for serum zinc and IgA measurements were not distributed normally for measured parameters, data for each treatment were compared to the control level with Wilcoxon rank test using

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proc NPAR1WAY, SAS software. Significance was declared at P<=0.05 and tendency towards significance was declared at 0.05<P<=0.15.

Based on the laboratory analysis of the orts animals actually received an average of 41.1, 58.4, 85.2, and 110.3 mg/kg DM Zn for groups that were designed to receive 40 (T40), 60 (T60), 90 (T90) and 120 (T120) mg/kg Zn, respectively.

Main scientific outcomes

DMI, milk production, milk composition and body weight change

Data for DMI, milk production and milk composition and body weight change are presented in table 1. For dry matter intake (kg/d), milk production (kg/d) milk fat and protein content (%), milk fat and protein yield (kg/d) there was no linear or quadratic trend and parameters did not differ significantly amongst treatments. Energy corrected milk (kg/d; ECM) also did not follow a linear or quadratic trend with respect to increase of dietary Zn and ECM was not different for experimental treatments. The difference in feed efficiency (FE; ECM(kg)/DMI(kg)) Body weight change (kg; initial body weight-final body weight, BWC) failed to reach significance for all the treatments and there was no linear or quadratic response to increasing amounts of dietary Zn for FE or BWC.

Table 2- Least square means of DMI, milk production, milk composition and body weight change for dairy cows in the experiment receiving incremental levels of dietary Zn

Parameter	Treatment[1]				*SEM*
	T40	T60	T90	T120	
DMI (kg/d)	25.1	26.9	28.1	27.7	0.68
Milk production (kg/d)	27.2	26.6	30.1	28.4	0.98
Milk fat (%)	3.85	3.88	3.79	3.86	0.06
Milk protein (%)	3.78	3.74	3.75	3.77	0.02
Milk fat (kg/d)	1.04	1.01	1.11	1.07	0.03
Milk protein	1.04	0.97	1.11	1.07	0.04
ECM (kg/d)	29.54	28.22	31.73	30.52	0.93
FE[2]	1.17	1.07	1.15	1.11	0.03
BWC (kg)[3]	43.5	44.5	38.6	53.4	5.24

Treatments: 0 = 40 mg/kg DM dietary Zn, T60 = 60 mg/kg DM dietary Zn, T90 = 90 mg/kg DM dietary Zn, T120 = 120 mg/kg DM dietary Zn

2: Feed efficiency = ECM(kg)/DMI(kg)

3: Body weight change = initial body weight-final body weight

Serum and Milk IgA and serum Zn Data for serum and milk IgA and serum Zn concentration are presented in table 3. Serum IgA concentration (ng/L) was the same for T90 and T120 (p>0.48) and strongly tended to be higher for T60 (P=0.06) compared to T40 with no significant linear nor quadratic trend. For milk IgA (ng/ml) content, there was no linear or quadratic trend and milk IgA (ng/ml) did not differ significantly for

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all the treatments compared to T40. Serum Zn content (mg/L) increased linearly with increase in the Zn content of diets (P=0.05) but there was no significant quadratic trend. Serum Zn content strongly tended to be higher for T90 (P=0.05) and tended to be higher for T120 (P=0.13) compared to T40 while the difference between T40 and T60 was not significant (P = 0.92).

Table 3. Serum and milk analysis performed in dairy cows in the experiment receiving incremental levels of dietary Zn (Mean±SD).

Parameter	Treatment[1]				*P* value[2]		
	T40	T60	T90	T120	C1	C2	C3
Serum							
IgA	226.5±78.72	288.3±107.67	235.8±127.2	244.7±81.73	0.06	0.87	0.48
Zn	0.82±0.09	0.81±0.133	1.02±0.187	0.95±0.134	0.92	0.05	0.13
Milk							
IgA	153.7±90.67	223.1±185.57	281.5±583.23	170.5±192.54	0.43	0.76	0.62

1: T40= 40mg/kg DM dietary Zn, T60= 60mg/kg DM dietary Zn, T90= 90mg/kg DM dietary Zn, T120= 120mg/kg DM dietary Zn

2: C1=T40 vs. T60, C2=T40 vs. T90, C3=T40 vs. T120

Health events

Mastitis was the most important health issue during the study. In fact, the incidence of mastitis during the study (33%) was unusually very high in comparison with our previous study (9%). The use of a not totally composted bedding material and arriving to the end of the rye-grass silage silo (which usually is mouldier) were the main causes that probably challenged the animals with their health events. Although no statistical analysis was done due to the low number of animals to evaluate health issues, cows in T120 had the lowest number of quarters with mastitis events.

Table 4. Incidence of clinical mastitis in dairy cows feed different doses of zinc supplemented with HiZox® as a feed supplement in the milking parlour. First row indicates the number of animals that had any mastitis event during the study. Then, Q0 means the number of healthy quarters within the mastitic animals, Q1 means the number of quarters that were treated once within the mastitic animals, Q2 means the number of quarters that were treated twice within the mastitic animals, Q3 means the number of quarters that became chronic within the mastitic animals.

	Treatments[1]			
	T1	T2	T3	T4
Animals	7	6	5	2
Q0	15	13	12	5
Q1	5	6	3	1
Q2	5	1	3	1

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Q3	3	4	2	1

[1]T1 = no Zn supplementation; T2 = Zn supplemented to have a final dose of 60 mg/kg DM; T3 = Zn supplemented to have a final dose of 90 mg/kg DM; T4 = Zn supplemented to have a final dose of 120 mg/kg DM

Conclusion

Zinc diet supplementation increased serum Zn concentration when Zn was supplemented at 90 and 120 mg/kg. Although no improvements in performance were observed, data from this study indicate slight benefits on health performance due the lower number of quarters with mastitis in Zn-supplemented cows compared with non-supplemented animals.

2.2 TNA 2

Objective

This study examined the effect of partial replacement (300 g) of corn meal with seaweed (*Ascophyllum nodosum*) on milk composition, efficiency, hematological parameters, rumen microbiome, and milk mineral composition. The hypothesis tested were that *Ascophyllum nodosum* seaweed was able to replace corn in lactating dairy cows diet without impairing performance and milk iodine content, and is able to alter rumen microbiome by reducing methanogens.

Material and Methods

Lactating Holstein cows (n = 48) were allocated into two experimental groups, balanced for parity, milk yield, contents of fat, protein, and somatic cell count (SCC), in a randomized block design. Diet treatments were (i) control (CON) and (ii) partial replacement of corn meal with *A. nodosum* (SWD; 330 g/day on dry matter (DM) basis). After a 2-week adaptation, cows consumed the experimental diets for 8 weeks. DM intake, feed, milk, and blood samples were collected daily, weekly, fortnightly and at the end of the trial respectively.

Also, rumen samples are under analyses for volatile fatty acids, ammonia, microbiome profile. Methane production was predicted according to VFA stoichiometry equation below:

$$Predicted\ CH4\ (ml) = 22.4 \times (0.5 \times AA - 0.25 \times PA + 0.50 \times BA - 0.25 \times VA)$$

where AA, PA, BA, and VA are the production (mmol) of acetate, propionate, butyrate and valerate, and 22.4 is the gas volume (ml/mmol gas) (Wolin et al., 1960)

Statistical analysis

Linear mixed models used diet, week, and their interaction as fixed factors, and cow (nested within diet) as random factor; with pre-treatment records as the covariate. Week was excluded from the model for hematological parameters (measured once).

Main scientific outcomes

Milk yield, basic composition and efficiency parameters were similar between CON and SWD. When compared with CON, cows fed SWD had lower neutrophil concentrations (2.65e9/L vs 2.06e9/L , p=0.009) and a tendency for lower white blood cell count (7.37e9/L vs 6.70e9/L, , p=0.050); although their concentrations were within the normal range for healthy dairy cows in both diets.

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Table 1. Means, standard error (SE), and ANOVA p-values for the effect of dietary treatment (Control, no seaweed, CON; Seaweed, 330g fresh per day, SWD) on animal diet data, milk production and basic composition, and efficiency parameters.

	Dietary Treatments			ANOVA p-Values [1]		
Parameters	**CON**	**SWD**	**SE**	**Diet**	**Week**	**Diet x Week**
Dry Matter Intake (kg/d)	24.54	24.53	0.368	0.979	0.000	0.000
Milk Yield (kg/d)	32.12	32.02	0.312	0.826	0.532	0.430
Milk Fat (g/100g milk)	3.65	3.64	0.060	0.899	0.000	0.528
Milk Protein (g/100g milk)	3.36	3.31	0.021	0.137	0.000	0.466
Milk Lactose (g/100g milk)	4.90	4.89	0.014	0.534	0.000	0.286
Solids non-fat (%)	8.98	8.93	0.027	0.207	0.000	0.318
Milk Urea (mg/L)	224.1	211.4	5.59	0.123	0.000	0.466
Milk SSC (x1000/ml) [2]	152.7	90.6	35.80	0.709	0.012	0.988
Fat:Protein	1.09	1.10	0.0249	0.574	0.008	0.617
ECMY [3]	31.62	31.40	0.7552	0.673	0.001	0.331
Protein Efficiency (g in milk/kg of DMI)	44.04	42.84	0.484	0.096	0.000	0.000
Fat Efficiency (g in milk/kg of DMI)	47.72	46.82	0.733	0.382	0.001	0.000
Feed Efficiency (kg milk/kg of DMI)	1.32	1.32	0.035	0.859	0.000	0.000
ECMY Feed (ECMY/kg of DMI)	1.31	1.27	0.020	0.070	0.000	0.000

[1] Significances were declared at $p < 0.05$.

[2] p-values were generated from the common logarithm of somatic cell count (SCC) values.

[3] Energy Corrected Milk Yield = milk yield (kg) × (0.01 + 0.0122 milk fat (g/kg) + 0.0077 milk protein (g/kg) + 0.053 milk lactose (g/kg))

Table 2. Means, standard error (SE) and ANOVA p-values for the effect of dietary treatment (Control, no seaweed, CON; Seaweed, 330g fresh per day, SWD) on final blood composition.

	Dietary Treatments			ANOVA p-Values [1]
Parameters	**CON**	**SWD**	**SE**	**Diet**
White Blood Count (10^9/L)	7.37	6.70	0.239	0.050
Neutrophils (10^9/L)	2.65	2.06	1.710	0.009
Lymphocytes (10^9/L)	4.36	4.33	0.203	0.932
Monocytes (10^9/L)	0.13	0.11	0.010	0.372
Eosinophils (10^9/L)	0.24	0.20	0.040	0.333
Basophils (10^9/L)	0.0003	0.0000	0.00035	0.330
Neutrophils (%)	35.9	30.7	2.06	0.060
Lymphocytes (%)	59.3	64.8	2.37	0.085
Monocytes (%)	1.71	1.70	0.146	0.958
Eosinophil (%)	3.083	2.957	0.418	0.650

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Basophiles (%)	0.0035	0.0000	0.00345	0.330
RBC (10^12/L)	5.90	5.70	0.117	0.211
Hemoglobin (g/dL)	10.42	10.38	0.158	0.829
Hematocrit (%)	28.84	28.59	0.449	0.681
MCV (fL)	49.14	50.36	0.804	0.212
MCH (pg)	17.78	18.31	0.295	0.146
MCHC (g/dL)	36.18	36.36	0.226	0.527
RDW-CV (%)	22.00	20.96	0.677	0.169
PLT (10^9/L)	246.2	231.1	23.30	0.631
MPV (fL)	6.52	6.56	0.108	0.801

[1] Significances were declared at $p < 0.05$.

Conclusion

This study provides evidence that corn meal can be safely partially replaced with *A. nodosum* at 330 g/day, without negative implications to cows' productivity, milk quality and efficiency, while changes in measured hematological parameters did not indicate any potential health risks.

2.3 TNA 3

Introduction

The laser methane detector (LMD), a hand held open path laser measuring device. Its measurements are based on infrared absorption spectroscopy using a semiconductor laser as a collimated excitation source. It employs second harmonic detection of wavelength modulation spectroscopy to establish methane concentration (Iseki, 2004). The LMD is manufactured by Tokyo Gas Engineering Solutions, Ltd. and was originally developed for the detection of gas leaks, and therefore, discriminate between high CH4 concentrations and the low background concentration in the atmosphere (Crowcon, 2017). Since its use in livestock methane determination was first introduced by Chagunda et al. (2009), different models of this device have been developed but using the same technology, namely tunable diode laser absorption spectroscopy. The LaserMethaneMini® is the one currently in circulation. Although different studies have so far employed the LMD to measure methane in both cattle and goats, some of the underlaying assumption in the measuring techniques which were proposed in earlier stuies (e.g. Chagunda et al., 2009; Chagunda et al., 2013; Chagunda, 2015) although based on sound biological knowledge, have not been fully tested.

Objective

The current study aimed at validating some of the major measuring assumption of the LMD in a systematic and robust manner. Specifically, the study investigated the effect of distance between the animal and LMD, monitoring angle, presence of adjacent animals on the reliability of the laser measurements and then identify the best combination to ensure a repeatable and reliable measure to quantify methane release by the animals.

Laser Methane m-g device measure methane concentration in ppm*m. Thus, we suppose that distance between the device and the animal should affect the concentration measured and should be corrected

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or fixed. When animal breath or belch, a cloud of air is exhaled and the methane concentration measured should also be affect by the size of cloud crossed by the laser beam. Thus, monitoring angle during the measurement should also affect the measurement. Finally, presence of adjacent animals should contaminate the concentration of methane measured if cloud of air exhaled by another animal is interfering with the cloud of air exhaled by the animal measured.
In this study, we tested the impact of these 3 factors on the reliability of the laser measurements and identified a protocol to ensure a repeatable and reliable measure to quantify methane release by the animals.

Materials and Methods

The study involved 20 lactating dairy cows of different age, number of lactation and milk production feeding with a unique ration established to cover the needs of dairy cows. In total, 720 LMm-g measurements (36 with each animal) of 4 minutes have been spread out over 20 days. The 12 combinations of distance from device to nostrils (2 and 3 meters), monitoring angle (45° and 90°) and distance between adjacent animals (0 gap, 1 animal width, 2 animal width between animals)) have been performed on each animal and repeated 3 times on the same day to assess the potential changes in methane emissions in relation to rumen fill (Figure 1). In practice, 3 different combinations measurements have been run 3 times on 4 animals each day (cf. table 2). Each combination factors was randomly selected for each animal per day and per measurement moment (Morning during feeding, Midday and Evening after milking). The four animals for each measurement moment have been choose randomly as described in the table 2. There is some practical considerations in this experimental design, for each measurement the presence order of adjacent animals was taken into account to better manage the experiments by operators (0 fence, 1 fence and 2 fences).

Table 1. Example of experimental plan for measurements of day 1.

			Measurement 1			Measurement 2			Measurement 3		
Order	Moment	Cow	Distances	Angles	gap	Distances	Angles	gap	Distances	Angles	gap
4	Morning	1	3	45	0	2	45	1	2	45	2
3	Morning	2	2	45	0	2	90	1	3	45	2
2	Morning	3	3	90	0	3	45	1	2	90	2
1	Morning	4	2	45	0	2	90	1	2	45	2
4	Midday	1	3	45	0	2	45	1	2	45	2
1	Midday	2	2	45	0	2	90	1	3	45	2
3	Midday	3	3	90	0	3	45	1	2	90	2
2	Midday	4	2	45	0	2	90	1	2	45	2
1	Evening	1	3	45	0	2	45	1	2	45	2
3	Evening	2	2	45	0	2	90	1	3	45	2
2	Evening	3	3	90	0	3	45	1	2	90	2
4	Evening	4	2	45	0	2	90	1	2	45	2

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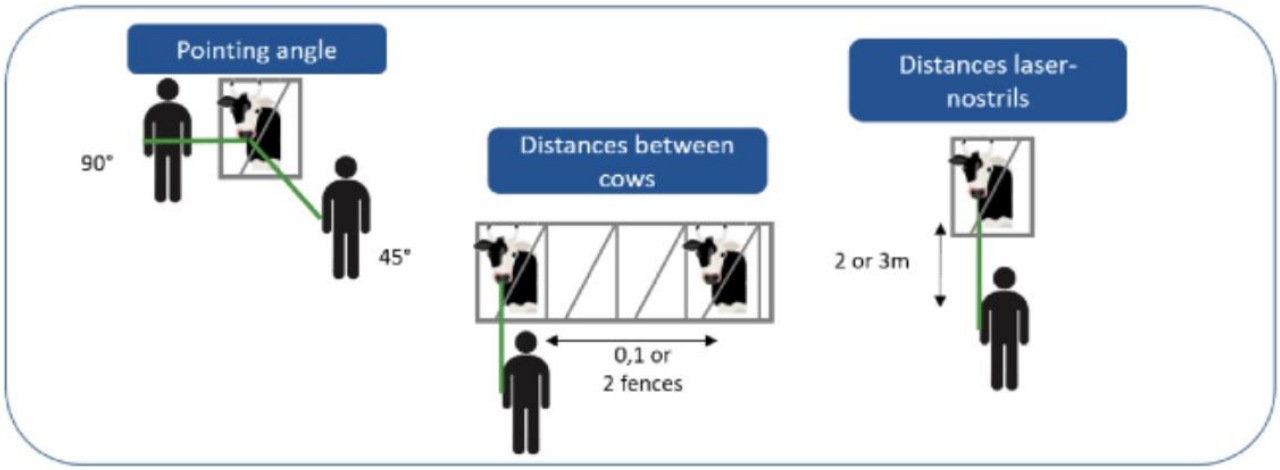

Figure 1 : Description of factors and modalities tested

Main scientific outcomes

Methane concentration measured are between 0 and 12 187 ppm*m (Table 2)

Table 2. Descriptive statistics of methane concentation (ppm*m)

Min.	Max.	Mean	Median	SD
0	12187	80.2	50	118.7

Methane concentrations are similarly distributed according to factors and modalities (Figure 2). Distance (2 or 3 meters), angle (45 or 90º) and proximity of neighbours (0 = 0 meter between cows, 1 = 1 meter between cows and 2 = 2 meter between cows).

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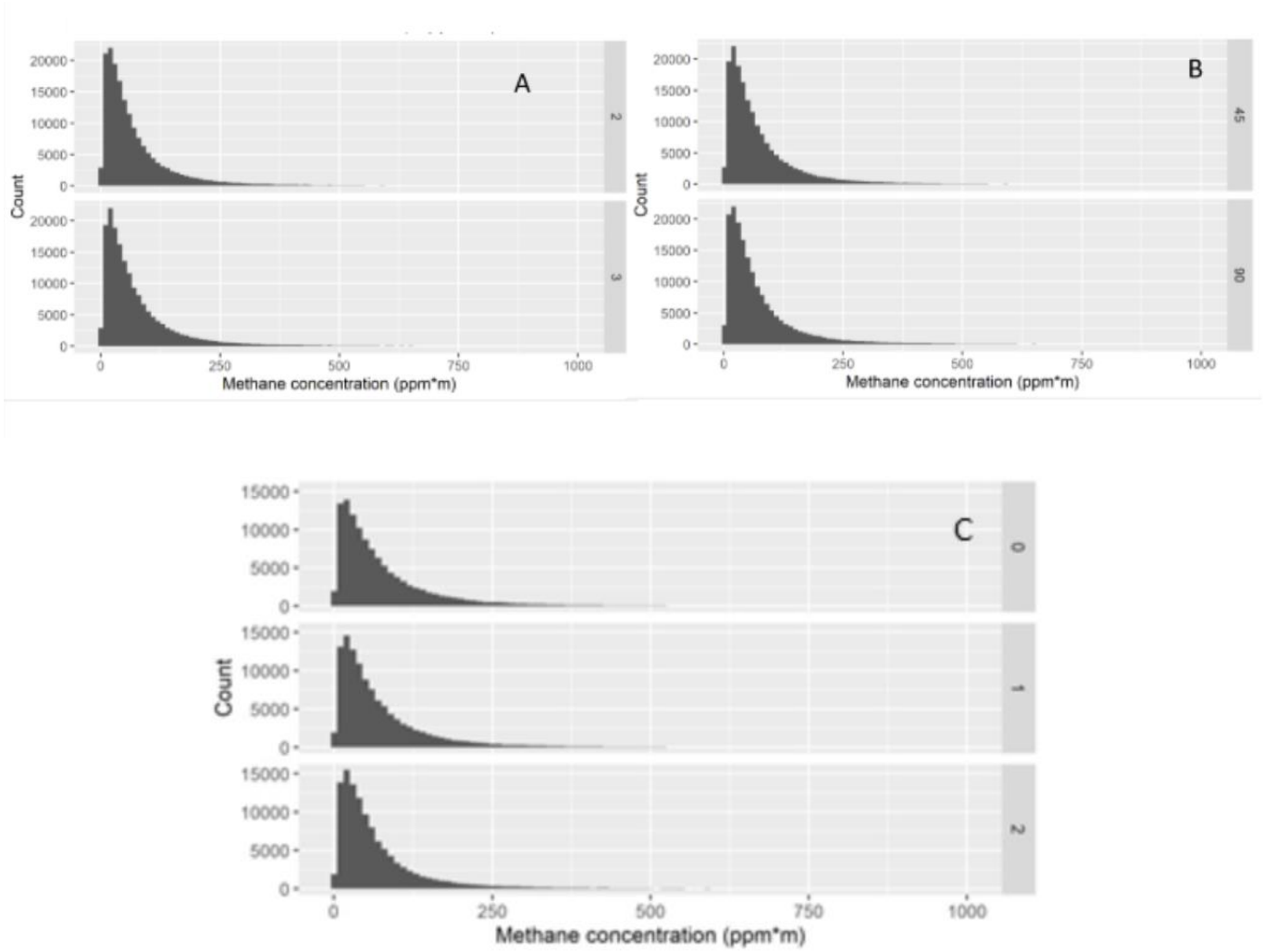

Figure 2. Distribution of methane concentration (in ppm*m) measured (A: Distance, B: angle, C. presence of neighbours.

Following boxplots report, PMEAN seems to be similar according to factors and modalities which average around 120 ppm*m. There were no significant differences between PMEAN factors.

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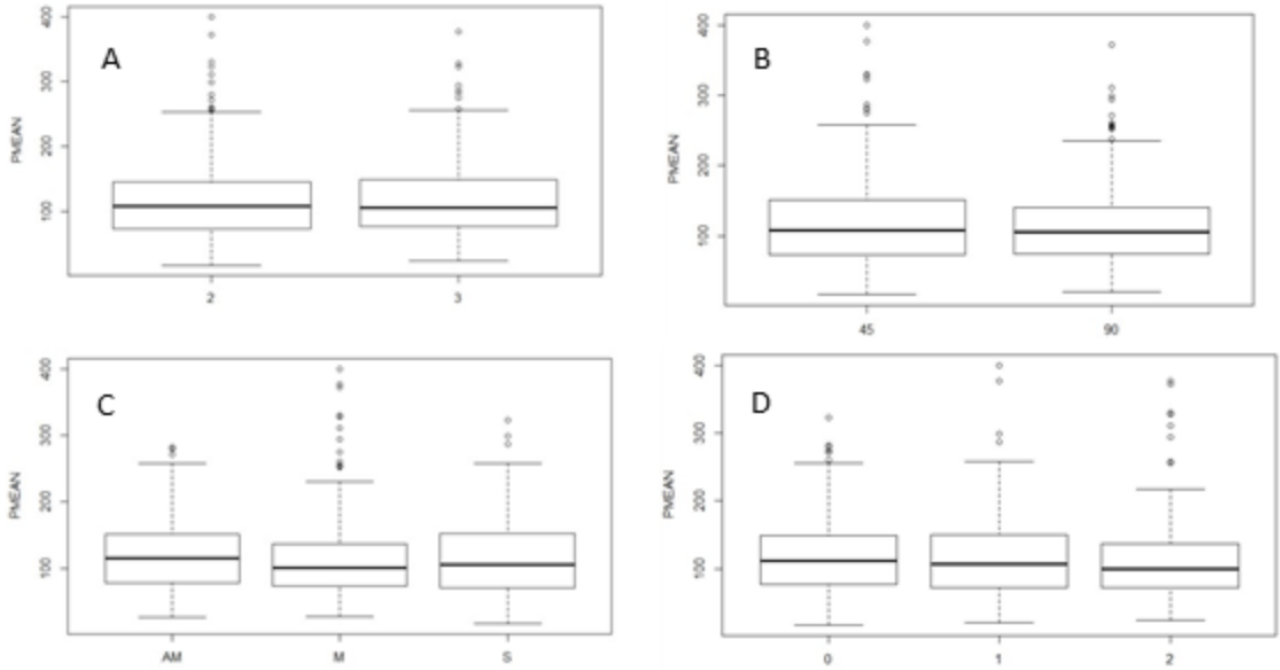

Figure 3. Distributions of PMEAN according to factors and modalities (A: distance, B: angle, C: moment of the day, D: presence of neighbours).

Conclusion

According to this trial, we can conclude that the position of operator during measurement did not affect the PMEAN value calculated from the 4 minutes laser measurement usually used on peer viewed scientific paper about laser.

Other variables are in study to be sure that the analysis did not miss some kind of difference between modalities of factors.

2.4 TNA 4

Objective

The objective of this study was to evaluate yeast supplementation in dairy cows diets.

The hypothesis was that yeast supplementation could improve rumen pH, regulate milk fat:protein ratio, and feed efficiency.

Materials and Methods

To assess this objective and to validate the hypothesis two groups of cows were done (n=18) one group with yeast supplementation in the concentrate feed delivered in the milking parlour twice a day, and the other without yeast supplementation. Animals were followed for 70 days after calving. During this period individual DM intake, milk yield and quality, body weight were recorded, and blood samples and BCS at 7, 28 and 70 day of study were obtained. Rumen pH was monitored in 7 animals per treatment throughout the study using rumen bolus (smaXtec, Graz, Austria).

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Results

No difference in performance nor feed intake were observed between treatments. However, it could be envisaged a more stable rumen pH when yeasts were included in dairy cows diets (Figure 1).

Table 1. Performance and milk composition data of dairy cows fed a supplement with (T1) or without (T0) yeasts.

	Treatments			P-values[1]		
	T0	T1	SEM[2]	T	time	Txtime
Initial BW	729	707	14.0	-	-	-
Final BW	684	672	14.0	0.57	<0.001	0.74
TDMI, kg/d	20.6	20.7	0.54	0.87	<0.001	0.97
Milk yield, kg/d	38.1	37.7	0.90	0.76	<0.001	0.96
Fat, %	3.55	3.43	0.083	0.30	<0.001	0.62
Protein, %	3.39	3.44	0.036	0.33	<0.001	0.99
ECM[3], kg/d	38.8	37.9	0.99	0.50	<0.001	0.94
ECM feed efficiency	1.96	1.90	0.052	0.40	<0.001	0.72
Milkoscan milk analysis,						
Fat, %	4.08	3.95	0.090	0.32	<0.001	0.54
Protein, %	3.03	3.03	0.051	0.98	<0.001	0.86
Lactose, %	4.96	4.92	0.025	0.25	<0.001	0.49
Total solids, %	8.77	8.74	0.053	0.65	<0.001	0.68
Urea, mg/L	184	184	5.0	0.98	0.0008	0.38
Log_{10} somatic cell counts	1.82	1.73	0.096	0.49	<0.001	0.10
BHBA, mmol/L	0.08	0.07	0.009	0.75	<0.001	0.28
Ratio fat:protein	1.35	1.32	0.029	0.36	<0.001	0.68

[1] T=effect of yeast; time=effect of day; Txtime = interaction of yeast supplementation with time

[2] Standard error of the mean

[3] Energy-corrected milk

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Table 2. Rumen pH data of dairy cows fed a supplement with (T1) or without (T0) yeasts.

	Treatments			P-values[1]		
	T0	T1	SEM[2]	T	time	Txtime
Average pH	6.74	6.71	0.094	0.82	0.02	0.58
Minimum pH	5.78	5.87	0.092	0.49	0.002	0.35
Maximum pH	6.22	6.26	0.084	0.74	0.04	0.34
Time < 5.8, min[1]	241	119	78.5	0.28	0.07	0.71

[1] T=effect of yeast; time=effect of day; Txtime = interaction of yeast supplementation with time

[2] Standard error of the mean

[3] Energy-corrected milk

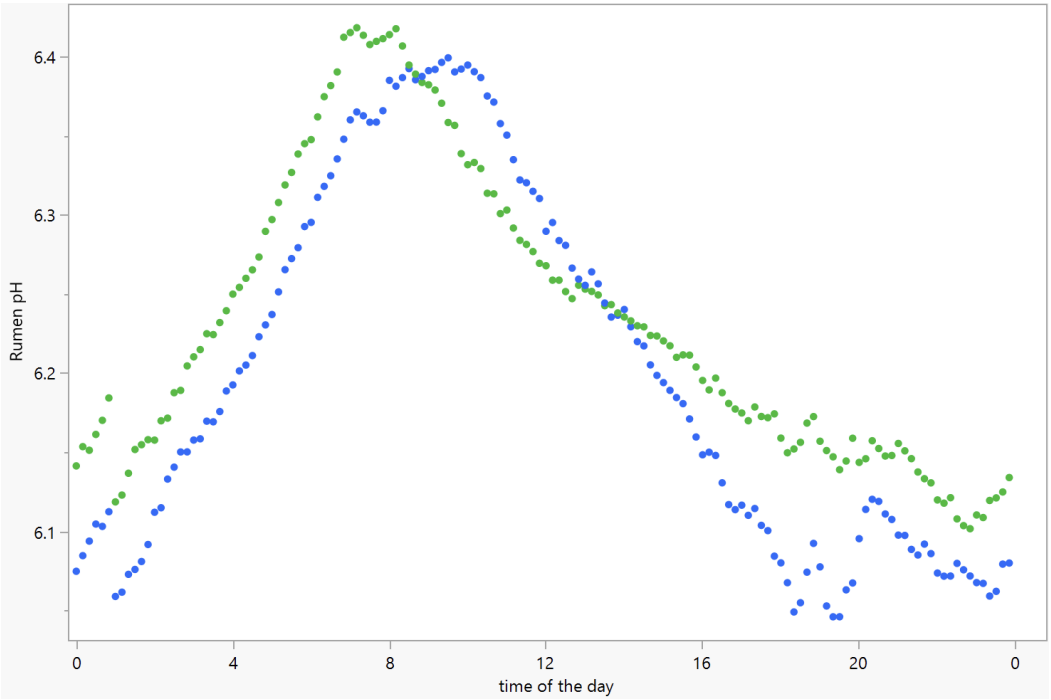

Figure 1. Daily evolution of average rumen pH by treatment every 10-min. In blue control cows and in green yeast supplemented cows are represented.

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