Journal of Animal and Feed Sciences, 28, 2019, 230–237 https://doi.org/10.22358/jafs/110413/2019
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Essential oil-bearing supplementation of dairy cows – *in vivo* experiments elucidating factors and co-factors influencing parameters of feed efficiency

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KEY WORDS: essential oils, feed additives, milk yield, phytogenic compounds, thymol

Received: 31 January 2019
Revised: 30 April 2019
Accepted: 17 July 2019

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ABSTRACT. This research aimed to elucidate effects of an essential oil (EO) supplementation including thymol, limonene and carvone as main active compounds on parameters of feed efficiency (FE) in early lactating Simmental cows. Two balanced groups of cows (n = 16) were used. The EO group received a total mixed ration (TMR) and a concentrate plus 0.56 g EO blend per kg of dry matter (DM), the control group (CON) was fed the same feed without EO dietary supplementation. Results showed higher DM intake (DMI) of TMR and daily milk yield for CON (22.48 and 39.17 kg) than for EO cows (21.03 and 37.10 kg, $P < 0.001$), while intake of the concentrate and FE (milk yield/DMI) did not differ. FE was increased as an effect of EO supplementation (1.84 vs 1.80, $P = 0.001$), if body weight and pre-experimental FE were respected as co-variates. The FE progress during the 4-week test period displayed a strong negative correlation of trend line slopes and intercepts (r = −0.887, EO group). An inherent FE of 1.69 separated the predicted FE progress by treatment. EO cows with a higher inherent FE proceeded in steeper FE slopes than CON. No evidence of a general increase of FE after EO intake in dairy cows could be determined, unless additional individual parameters are considered.

Introduction

Various publications highlight the effects of phytogenic compounds and essential oil (EO) based feed additives in ruminants, though the results are controversial. Santos et al. (2010) found that an essential oil mixture (EOM) decreased dry matter intake (DMI) in dairy cows, but did not affect milk yield, while milk fat content increased. Giannenas et al. (2011) demonstrated an increase in milk production in ewes after EO supplementation and Benchaar et al. (2006) suggested that EOs have the potential to increase feed efficiency (FE) in beef cattle. Tager and Krause (2011) found that EOM dietary inclusion did not affect DMI, milk yield and composition in lactating dairy cows. Due to the volatility of EO compounds, EOMs in feed can affect taste and, in consequence, palatability of feed, as well as appetite of farm animals. Benchaar and Greathead (2011) mentioned that the effective dose of EOM in feed might negatively affect the palatability. Apart from the aforementioned effects, EO compounds could enhance stress resilience in dairy cows and thereby stabilize milk yield. Furthermore, they could serve as alternatives to banned feed antibiotics, which were used as promoters of the animal performance (Hashemzadeh-Cigari et al., 2015; Kim et al., 2016).

Nowadays, the *in vivo* effects of EO supplementation on parameters of feed efficiency (FE, milk yield/DMI) in dairy cows are of great importance, as rising global competition forces agriculture to increase efficiency to maintain profitability (Socha et al., 2007). While *in vitro* results provide evidence that EOMs have the potential to improve energy utilization in ruminants by positively shifting the propionate:acetate ratio in the rumen, *in vivo* results are inconsistent (Thao et al., 2014; Dai et al., 2017). One of the reasons for the lack of consistent positive *in vivo* effects might be the adaptation of rumen bacteria to EO in long-term applications (Benchaar et al., 2008). Obviously, varying results reveal a certain unpredictability of *in vivo* EO feeding success (Patra, 2011; Kim et al., 2016). Pre-determined co-factors like inherent FE or milk yield, period of lactation, body weight (BW) or breed seem to affect the observed effects of EOs (Tekippe et al., 2013; Drong et al., 2016). Furthermore, other details like varying composition of phytogenic material, varying EO content, method and dose of intake, preparation of EO bearing feed material, possible interactions with the basal diet, selection of certain EO compounds, may influence the effects on FE (Spanghero et al., 2009; Tager and Krause, 2011).

This research focused on elucidating *in vivo* animal related effects of a provided EO bearing supplement consisting of thyme (*Thymus vulgaris*) and caraway (*Carum carvi*) as main plants on parameters of FE in dairy cows by taking pre-existing factors into consideration. The aim was to work towards predictability of effects of EOs as feed additives on FE by additionally considering *in vivo* co-factors.

Material and methods

The research project was discussed and approved by the institutional ethics committee in accordance with Good Scientific Practice (GSP) guidelines and national legislation.

Cows, diets and experimental design

This research comprised a feeding trial with 16 early lactating Simmental cows divided into two groups of 8 cows in each. Total number of cows complied with comparable trials assessing dairy cow performance (Khol-Parisini et al., 2016; Lejonklev et al., 2016). The cows in our research were in their 2nd to 4th lactation, except one cow in its 7th lactation. Mean lactation of all cows was 2.88; the average daily milk production amounted for 35 to 40 kg milk per day. Body weight (BW) of all cows was assessed on a due date directly before the experiment started and ranged from 692 to 940 kg, with a mean of 826.5 kg. Dry matter intake (DMI) of total mixed ration (TMR) at the last week before the beginning of the experiment ranged from 15.11 to 26.11 kg/day, with a mean of 20.92 kg/day, and the respective feed efficiency (FE) of TMR ranged from 1.52 to 2.57, with a mean of 1.79.

Table 1. Pre-experimental body weight (BW), ordinal number of lactations, dry matter intake (DMI), milk yield and feed efficiency (FE) in control (CON) and treated[1] (EOM) groups

n[2]	Indices	CON		EOM		*P*-value
		mean	SD	mean	SD	
16	BW, kg	799	64	854	59	0.095
16	Lactation	2.75	1.75	3.00	0.54	0.705
16	DMI (TMR), kg/day	22.11	2.95	19.73	2.74	0.117
13	Milk yield, kg/day	38.34	2.83	36.21	3.08	0.219
13	FE (TMR)	1.74	0.21	1.86	0.37	0.476

[1] treated cows were daily supplemented with 2–3 g of powdery plant material mainly consisting of thyme and caraway; [2] number of collected values for the respective trait; TMR – total mixed ration

Groups were balanced for BW, lactation, 1-week pre-experimental DMI, milk yield and FE (Table 1).

The experiment was conducted at the research farm ‘Kremesberg’ of Vetmeduni, Vienna (Austria), during a period of five months in the first half of the year 2015, within which parturition in the 16 test cows took place. During the experiment, the cows were kept together in a loose-housing stable with bedding.

For each cow, the individual test period lasted 6 weeks with the 2 weeks of adaptation period and 4 weeks of measurement. Two weeks after calving, cows were alternately allocated to two feeding groups: the untreated (CON) and treated group EOM); n = 8 per group.

Throughout the experiment and in the pre-experimental adaptation period, all cows were fed a total mixed ration (TMR) with maize and grass silage as the main ingredients (Table 2). An additional amount of concentrate (Table 2) was provided to the cows according to their milk production with a daily maximum allowance of 5.35 kg dry matter (DM, 6 kg as fed). Cows had continuous access to feed and fresh water. Daily intake of TMR and

Table 2. Analysed chemical composition of total mixed ration (TMR)[1] and concentrate[2], % of dry matter (DM), unless stated

Indices	Total mixed ration	Concentrate
DM, %	42.28	89.20
NEL[3], MJ/kg	6.20	7.00
Crude protein	13.50	20.98
Crude fat	2.06	3.54
Neutral detergent fibre	35.05	22.95
Acid detergent fibre	24.58	12.30
Non-fibre carbohydrate[4]	42.86	46.78

[1] ingredients of the TMR in % of DM: grass silage (2nd cut) 36.89, maize silage + grass silage 42.57, grain mix 15.45, protein supplement 2.6, hay 1st cut with clover and herbs 2.5; [2] ingredients of the concentrate mixture, as given by the producer in % of DM: rapeseed meal 19, maize 17.5, triticale 15, maize concentrate 13.5, dried distillers grains with solubles (DDGS) 12.70, wheat 8.8, wheat bran 7.2, molasses 4, calcium carbonate 1.4, cocoa shells 0.5, cattle salt 0.2, premixes 0.2; [3] NEL – net energy content for lactation; [4] calculated: 100 − (crude ash + crude protein + crude fat + neutral detergent fibre)

concentrate was measured electronically (Roughage Intake Control System, RIC, Hokofarm Group, Marknesse, Netherlands and DeLaval Feed Station, DeLaval, Tumba, Sweden) for each cow throughout the experimental period.

The treated group was supplemented with 2–3 g EOM daily, as recommended by the supplying company. The EOM was provided through the concentrate (conc.) in a concentration of 0.5 g EOM/kg conc. as fed (0.56 g EOM/kg DM). The offered phytogenic supplement consisted mainly of powdery plant material of thyme (*Thymus vulgaris*) and caraway (*Carum carvi*), providing the main bioactive EO compounds such as thymol, limonene and carvone. The supplement was mixed into the concentrate twice during the five months of experimental period. The ingested amount of EOM was calculated *via* the measured amount of consumed concentrate per cow and day. The mean daily intake of the feed additive amounted for 2.89 ± 0.55 g for the treated cows.

Cows were milked twice a day in the morning and in the evening and the daily milk production was recorded. For 14 out of the 16 cows, milk fat and protein percentages, analysed by Fourier-transform infrared spectroscopy (FTIR, CombiFoss™ 7, Foss GmbH, Hilleroed, Denmark) of one representative sampling, were available. The sampling took place within the standard cattle milk recording for quality control of the herd, according to the standards of the International Committee for Animal Recording (ICAR) and was conducted by the Qualitätslabor Gmünd, for LKV Niederösterreich (Zwettl, Austria).

Data of feed intake (TMR and concentrate) and milk production were individually collected daily for 28 consecutive days. FE was calculated on the base of dry matter intake (DMI) of TMR and of total DMI (TMR and concentrate), respectively, and of actual milk yield. In addition, daily 4% FCM yield and ECM was calculated and presented.

Feed efficiency (FE), 4% fat corrected milk (FCM) and energy corrected milk (ECM) were calculated according to the following equations (Gruber et al., 2014; Münnich et al., 2017):

$$\text{FE} = \text{daily milk yield (kg/day)} / \text{daily DMI (kg/day)} \quad (1)$$

$$\text{FCM} = \text{milk yield (kg)} \times (0.4 + \text{milk \% fat} \times 0.15) \quad (2)$$

$$\text{ECM} = (0.38 \times \text{milk fat \%} + 0.21 \times \text{milk protein \%} + 0.95) \times \text{kg of milk} / 3.2 \quad (3)$$

Analysis of feed

Individual TMR ingredients and concentrates were sampled monthly for chemical composition analysis and the average values of all samplings are reported (Table 2). The feed samples were analysed for the proximate nutrient composition (VDLUFA, 2007). Prior to analysis, samples were oven-dried at 50 °C for 48 h and ground passing through a 0.75-mm sieve. The ground materials were randomly sampled for determination of dry matter (DM), organic matter (OM), crude protein (CP), crude fat, acid detergent fibre (ADF) and neutral detergent fibre (NDF). Dry matter was analysed by oven drying at 100 °C over night and ash by combustion of samples at 580 °C over night. Organic matter was subsequently calculated from the dry matter and ash content. Crude protein was analysed according to the Kjeldahl's method (VDLUFA 2007). The content of ADF and NDF was determined using the Fiber Therm FT 12 (Gerhardt GmbH & Co. KG, Königswinter, Germany). For NDF determination heat-stable α-amylase was added. The ADF and NDF values were reported exclusive of residual ash. Crude fat was analysed as ether extract using a Soxhlet extractor (Extraction System B-811, Büchi, Flawil, Switzerland).

Statistical analysis

All statistical analyses of *in vivo* data were performed with IBM SPSS Statistics ver. 23 software (IBM, Armonk, NY, USA). All significances were declared at the probability of error level of α = 0.05, unless otherwise stated.

The single cow was the experimental unit. The effects of EO supplementation, as single factor as well as in covariance with pre-experimental co-factors, on daily DMI, daily milk yield and composition and calculated FE, and on progress of all variables during the measuring period were examined by using the general linear model. Linear regression was calculated for the actual daily EOM intake as predictor for FE (TMR).

The progress of variables was expressed as linear trend estimation, showing data as a linear function of time. Trend lines of daily TMR (DM) intake, milk production and FE during the test period were plotted with the respective trait as y-axis and the elapsed time as x-axis and analysed as a least squares regression function. The equations of the linear trend lines were of the form

$$y = kx + d \quad (4)$$

where: y – dependent variable, k – slop of the line, x – explanatory variable and d – intercept (intersection with the y-axis). Slopes and intercepts were used to characterize the progress of the variables.

Possible effect of sampling day on measured traits was checked by analysis of variance.

As cow's individuality may play a crucial role for FE reaction to EOM supplement, the individual slopes and intercepts of the trend lines of FE, DMI and milk yield were displayed and analysed graphically. For this purpose, slopes or intercepts of the respective trend lines of variables of interest were exhibited in scatterplots. Where appropriate, the association of two variables was expressed as correlation coefficient, linear regression lines were fitted to observed data and the linear coefficient of determination was used to check the goodness of fit for the observations within both treatment groups.

Results

Analysis of variance / influence of treatment

Daily TMR (DM) intake was decreased by supplementation of EOM (21.03 and 22.48 kg, for treated and CON cows, respectively, $P < 0.001$). Mean values of daily concentrate (DM) intake did not show any differences due to EOM supplementation. In comparison to control diet, the EOM supplementation decreased ($P < 0.001$) total DMI (TMR + concentrate) from 27.50 to 26.21 kg/day (Table 3).

As compared to control diet, the EOM supplementation decreased actual daily milk yield from 39.17 to 37.10 kg ($P < 0.001$). Neither FCM and ECM yields vary due to supplementation of EOM, nor did milk composition of fat and protein. FE (TMR) and total FE (TMR + concentrate) were also not affected by treatment (Table 3).

Table 3. Influence of treatment[1] (univariate general linear model) on *in vivo* parameters of feed efficiency of lactating Simmental cows, for 4-week test period (mean values, regression parameters of the progress and standard deviation (SD))

Indices	n	CON		EOM		*P*-value
		mean	SD	mean	SD	
DMI, kg/d						
TMR	430	22.48*	3.53	21.03*	3.26	<0.001
concentrate	371	5.10	1.15	5.23	0.85	0.193
total (TMR + conc.)	358	27.50	3.41	26.21	3.25	< 0.001
Milk yield, kg/d						
actual	423	39.17*	4.68	37.10*	3.32	< 0.001
4% FCM	14	42.20	4.26	39.39	8.80	0.488
ECM	14	40.68	3.25	37.95	6.78	0.384
Milk composition, %						
fat	14	4.65	1.79	4.39	1.31	0.76
protein	14	3.12	1.29	3.08	0.31	0.802
FE						
TMR	406	1.78	0.30	1.81	0.32	0.304
total (TMR + conc.)	341	1.44	0.22	1.44	0.22	0.988
Progress DMI (TMR)[2]						
slope	16	0.08	0.14	0.04	0.10	0.502
intercept	16	21.27	3.28	20.48	3.44	0.645
Progress milk yield[2]						
slope	16	−0.03	0.14	0.03	0.10	0.318
intercept	16	39.55	4.54	36.72	2.57	0.146
Progress FE (TMR)[2]						
slope	16	−0.008	0.01	−0.004	0.01	0.512
intercept	16	1.88	0.25	1.86	0.31	0.889

[1] treated cows were daily supplemented with 2–3 g of powdery plant material mainly consisting of thyme and caraway; [2] progress of relevant trait during the 4-week measuring period; CON – control group; EOM – treated group; DMI – dry matter intake; TMR – total mixed ration; FCM – 4% fat corrected milk yield; ECM – 4% fat, 3.4% protein, energy corrected milk; FE – feed efficiency, based on actual milk yield; BW – body weight; conc. – concentrate, n – number of collected values for the respective trait; * – indicates significant differences in the same row at a level of probability of α = 0.05

By analysing the daily intake of EOM as a linear predictor for TMR FE, the following regression equation was found:

$$\text{FE (TMR)} = 0.130 \times \text{daily EOM intake} + 1.438 \quad (5)$$

Following this equation, 0 g EOM daily would cause a FE of 1.44, 2 g of EOM daily a FE of 1.70 and 3 g EOM daily a FE of 1.83. R^2 was 0.05 ($P = 0.003$), providing a significant predictor and 5% predictability on a linear basis.

Neither slopes, nor intercepts of trend lines of daily DMI (TMR), milk production and FE (TMR) varied due to EOM treatment during the experiment.

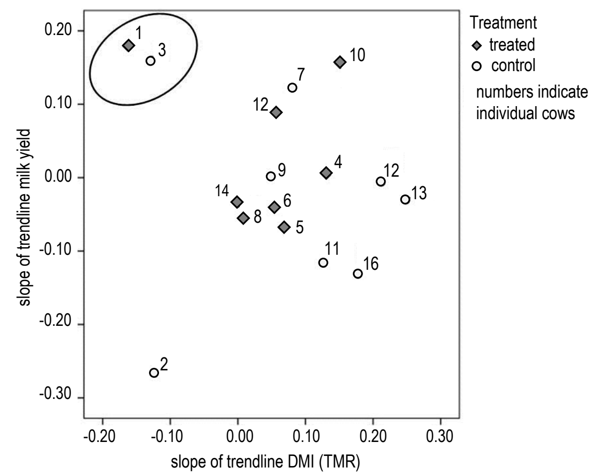

Figure 1. Scatter plot of all cows indicated by their treatment and number and distributed according to the slopes of trend lines of daily milk yield and of daily TMR (DM) intake during the test period

The circled and separated position of two cows indicates the desired progress of feed efficiency, resulting from an increase in milk production (positive slope) and a decrease in DMI (negative slope); TMR – total mixed ration; DM – dry matter; DMI – dry matter intake

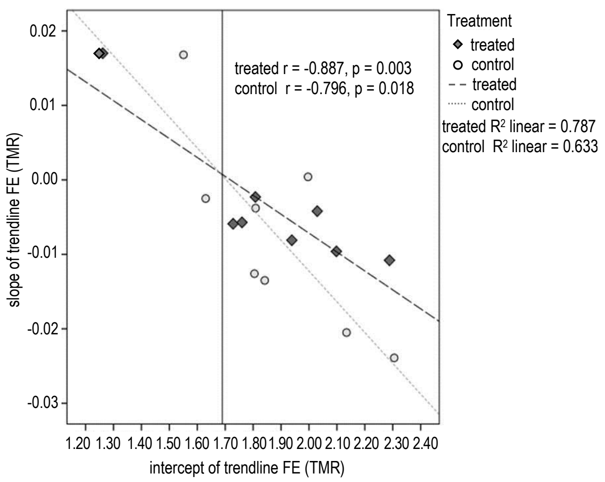

Figure 2. Bivariate correlation of slopes of feed efficiency (FE) of total mixed ration (TMR) trend lines during test period and interpolated starting point of FE (intercept) among both treatment groups, including linear coefficient of determination (R^2), coefficient of correlation (r) and *P*-values (two-tailed significance of correlation (Pearson))

The values showed a general parallelism of both treatment groups and were generally higher for the CON group, apart from a slightly negative slope for milk yield in CON cows (−0.03) vs a positive slope in treated cows (0.03, Table 3).

Periodicity or effect of sampling day during the test period could be excluded, showing *P*-values of 0.888, 1.000 and 0.643 for DMI (TMR), milk yield and FE (TMR), respectively.

Analysis of correlation / trends, slopes and intercepts

A scatterplot of the individual slopes of the trend lines of DMI and milk yield separated the single cows and revealed an unexpected separation, not by treatment, but by desired FE progress during the test period (negative slope in DMI and positive slope in milk yield). Among the two separated cows one (cow 1) was a treated cow with the highly positive slope of the FE trend line of 0.017 (Figure 1). Slopes of the FE trend line in the whole experiment ranged from −0.024 to 0.017 with a mean of −0.006. A similarity of the two separated cows was the high BW of 940 and 876 kg, respectively.

Among the treated cows, the correlation of intercepts of the individual FE trend lines during the experimental period, ranging from 1.26 to 2.29 (mean 1.87) and the slopes, ranging from −0.024 to 0.017 (mean −0.006), provided a very strong, negative correlation of r = −0.887 and a high linear (R^2 = 0.787) coefficient of determination. Cows of the control group developed a curve with a linear R^2 of 0.633 and the r = −0.796 (Figure 2). Therefore, a lower basic FE (intercept) will result in a steeper improvement of FE in the specific early lactation period (slope), which was true for both treatment groups. Nevertheless, there was an intersection of curves of treated and control group at an intercept of 1.69. Above the intercept of 1.69, the treated group developed steeper FE slopes than the control group; beneath the point of 1.69 the control group proceeded in steeper FE slopes (Figure 2).

Analysis of covariance / influence of body weight and pre-experimental values

When including BW and 1-week pre-experimental FE as co-factors, treatment turned out to be a significant main factor that affected mean FE during the test period (1.84 for EOM group vs 1.80 for CON group, *P* = 0.001). In this case, BW was a strong co-factor (*P* = 0.001) and pre-experimental FE provided no influencing strength (*P* = 0.403, Table 4). The direct correlation between BW and FE in the treated group was low and negative (r = −0.249).

Pre-experimental milk yield was a significant co-factor (*P* = 0.002, Table 4) that caused an effect of EOM supplementation on milk yield, indicated by the different treatment effects without and with this co-factor (*P* < 0.001, Table 3 and *P* = 0.088, Table 4, respectively). The correlation

Table 4. Analysis of covariance for the main dependent variables in test period (dry matter intake (DMI), milk yield and feed efficiency (FE)), including the main factor (treatment) and the pre-experimental cofactors (body weight (BW) and the respective variable one week prior to test period)

n	Dependent variable	Main factor	*P*-value	1. Cofactor	*P*-value	2. Cofactor	*P*-value
16	mean DMI (TMR) – test period	treatment	0.140	BW	0.247	mean DMI (TMR) – pre-experimental	0.228
13	mean milk yield – test period	treatment	0.088	BW	0.266	mean milk yield – pre-experimental*	0.002
13	mean FE (TMR) – test period	treatment*	0.001	BW	0.001	mean FE (TMR) – pre-experimental	0.403

n – number of collected values for the respective trait; TMR – total mixed ration; * – indicates significant influence on the dependent variable at a level of probability of α = 0.05

between FE during test period and pre-experimental milk production within the treated group was positive on a medium level (r = 0.588), the correlation between pre-experimental milk yield and milk yield in the test period in the treated group was very high and positive (r = 0.982, two-tailed significance on level 0.01).

Discussion

Essential oils bearing phytogenics are natural and granted as safe (GRAS) feed additives that could enhance FE, and therefore have gained great interest in recent years. The limitation for their extensive use is the unpredictability of their activity. Several factors that can be used for reliable prediction are examined in the present study.

Crucial for the improvement of predictability are the knowledge of specific fed EO compounds and their mode of action in ruminants. *Thymus vulgaris* (including thymol) was shown to inhibit the growth of potential pathogens and thereby relieves the animal from immune defence stress (Hashemi and Davoodi, 2011). *Carum carvi* (including limonene and carvone) is often mentioned in terms of milk yield enhancement (Sedláková et al., 2001). Reports of the effect of certain compounds like thymol and limonene or a mixture of them show a higher fat content in milk without increasing total milk yield (Reza-Yazdi et al., 2014). This finding is in agreement with that of Moheghi et al. (2010) that offered caraway supplemented diets in dairy cattle. In our findings neither fat, nor protein content of milk showed differences between treatment groups (P = 0.76 and 0.802, respectively), but the effect might also be dose-dependent (Lejonklev et al., 2016). *In vitro* analysis showing anti-bacterial activity of bioactive compounds leads to changes in rumen microbiota, tending to decrease acetate production and/or the acetate:propionate ratio of volatile fatty acids (Castillejos et al., 2006; Klevenhusen et al., 2012). As propionate can be effectively used as an energy source for ruminants, this effect *in vivo* can support milk production (Klevenhusen et al., 2012). Our *in vivo* results showed that after the calculation of 4% FCM instead of actual milk yield the significant negative influence of EOM was removed (P = 0.488 vs P < 0.001). The same trend was observed for ECM (P = 0.384 vs P < 0.001). Generally, reports about the efficacy of EOM to increase milk fat content are inconsistent or negative (Drong et al., 2016).

The elucidation of the *in vivo* co-factors, e.g. cow individual and pre-existing factors, is the greatest challenge in terms of predictability of success.

Firstly, composition and dose of EOM could affect palatability and appetite (Chapman et al., 2016). In our study, the EOM supplying company recommended a daily intake of EOM per cow in the range of 2–3 g, to ensure feasible handling by practitioners. By mixing the EOM to the concentrate in a ratio of 0.5 g/kg concentrate as fed and the daily allowance of 6 kg concentrate per cow, this intake could be guaranteed. Calculated *via* the actual taken-in concentration, a mean of 2.89 g EOM daily occurred. The dose was chosen on the one hand to have a likely positive effect on performance (Benchaar et al. (2008) reported of effective inhibition of amino acid deamination and therefore, improvement of rumen N and energy utilization by the daily addition of 1 g EOM/cow, containing thymol as one of the main active compounds) and on the other hand to avoid a loss of palatability of feed due to volatile taste-modulating compounds (Calsamiglia et al., 2007; Benchaar et al., 2008). As concluded by the significantly lower mean values of TMR intake in the treated group in our study, the desired mode of action of the additive (increase of feed intake by stimulating appetite) was not confirmed. At the same time, no significant differences between treatments concerning the concentrate intake were found. Therefore, the additive – provided at a dose of 2–3 g daily through the concentrate – did not increase the appetite (rather the opposite) and left the palatability unchanged. Other research on ruminants fed comparable EO supplements revealed no alteration in DMI, even at a higher dosage

(0.4 g daily for 17 kg lambs (Malekkhahi et al., 2015); 0.25–10 g daily for lactating dairy cows (Tager and Krause, 2011)). Nevertheless, further research is necessary to clarify these discrepancies in the values of TMR intake after EO supplementation.

Also, pre-existing FE (exhibited as the intercept of the FE trend line during test period) was shown to be strongly negatively correlated with the slope of FE reaction in treated cows. In consequence, it can be assumed that the inherent FE of a cow is one of the important pre-existing factors, which can strongly influence the efficacy of an EOM supplementation. It seems that a higher probability of cows with low starting FE to benefit from EOM feeding exists. Nevertheless, results indicate that there is a certain point of inherent FE (1.69 in our study), separating the benefit of FE increase for treated vs untreated cows. EOM treated cows with a higher inherent FE as 1.69 will rather react with a stronger FE increase than cows without EOM supplementation. Below this critical point, EOM feeding might not result in the same advantage in the early lactation period.

As shown by the analyses of covariance, BW and pre-experimental milk yield also can be strong co-factors affecting FE after EOM intake. Without taking BW into consideration, treatment with EOMs showed no influence on FE (TMR), but the effect is significant in case of BW inclusion in the model. Still, within the treated group, the direct correlation of BW and FE was low and not significant, indicating other additional influencing factors. In our experiment, treatment negatively influenced actual milk yield, but by taking pre-experimental milk yield into consideration, this co-factor revealed significant influence on experimental milk yield and in this case the effect of treatment was not notable. It is therefore essential, without neglecting pre-existing and influencing co-factors like BW and milk yield, to evaluate *in vivo* effects of EOM on FE.

Conclusions

In our experiment an essential oil mixture (EOM) including thymol, limonene and carvone at a daily dose of 2–3 g was used. Considering our results, no direct and general influence of EOMs on feed efficiency (FE) can be stated. Variability, caused by additional *in vivo* conditions, was high and cannot be easily controlled. Further *in vivo* research with a focus on constant content, quality and concentration of EOMs, as well as on the effects of individual co-factors like body weight and inherent FE and milk yield is appreciated to improve predictability of EOM effects.

Acknowledgements

This work was supported by the Austrian Research Promotion Agency (FFG) (Vienna, Austria). The authors thank Dr Werner Pohl, Angelika Kaefer and Elmar Draxler at the Teaching and Research Farm of the University of Veterinary Medicines (Vienna, Austria) for their comprehensive organizational and technical help. Without the extraordinary equipment, the facilities and the know-how at the research farm, implementation of the project would not have been possible. Furthermore, the authors thank Dr Khiaosa-Ard from the Institute of Animal Nutrition at the University of Veterinary Medicines (Vienna, Austria) for analysing and calculating the ingredients of the total mixed ration. Many thanks also to a company providing a suitable essential oil blend for this research.

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