Report on

Effects of dietary yeast supplementation on milk production, feed intake, fecal, and urinary variables in dairy cows under field conditions in Denmark

Yeast products tested

ChemVet dk A/S (Lesaffre, Actisaf Sc47) and Biomin Holding GmBH (Levabon Rumen E)

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BACKGROUND

Yeast products are marketed to Danish dairy farmers based on several claims. These include rumen pH management, fiber digestion, increased productivity, health claims mainly related to prevention of SARA (Sub Acute Ruminal Acidosis), improved milk quality, reproduction effects etc. In general these claims do not appear to be backed by studies applying to common feeding practices in the Danish dairy industry.

Extension offices associated with the Danish Agricultural Advisory Service report that relatively few of their dairy customers supplement dairy diets with yeast (approx. 5%). The fraction of diets optimized for minerals and vitamins in collaboration with pre-mixers is considerably higher (approx. 30%). It appears, therefore, that yeast supplementation is an option easily implemented in many more diets compared to the present situation.

The background for conducting a field study of yeast supplementation using two Dairy Clusters (experimental platform “kvægklynger”) was to the need for better evaluation of the production potential for increased use of yeast supplementation in Danish dairy diets.

OBJECTIVES

The objectives of the present experiment was to study effects of yeast supplements under field conditions in Denmark in an experimental setup based on two clusters of dairy farms and using dairy farm as the experimental unit. Response variables were: efficiency control data (feed intake, milk production, nutrient utilization for milk production); test-day milk recording data (milk yield, milk composition, and somatic cell count); fecal composition and fecal fiber digestibility; and urinary markers for nutritional and physiological status.

MATERIALS AND METHODS

Product inclusion in study by invitation:

In the period June to October 2012, Knowledge Centre for Agriculture invited 4 suppliers of yeast products to the Danish dairy industry (including both existing and potential suppliers), to participate in the study, and presented the overall experimental plan for the trial.

Two suppliers, assumed to have relatively market shares on the Danish market for yeast products declined the invitation to participate in the study, see table below. Two suppliers accepted the invitation to participate in the study. Participating suppliers sponsored the yeast and placebo products fed in the study and covered the costs related to fecal fiber analyses. Costs related to maintenance of the experimental platform were covered by grants held by Knowledge Centre for Agriculture, Denmark.

Invited supplier (product)	**Contact methods**	**Response to invitation**
ChemVet dk A/S (Lesaffre, Actisaf Sc47)	Email and phone contact	Accepted invitation to participate in study
Biomin Holding GmBH (Levabon Rumen E)	Personal contact, email, and phone	Accepted invitation to participate in study
Lallemand (LEVUCELL-SC)	Phone contact to Lallemand Nordic and email and phone contact to Lallemand Animal Nutrition, France	Initially interest for participation, but the company finally declined to participate in the study
Alltech (Yea-Sacc)	Personal contact to Danish distributor, Vitfoss, Gråsten, Denmark Email contact to Alltech, USA Email and phone contact to Alltech Denmark, Vejle, Denmark	Participation declined by Vitfoss, Denmark No response to proposed participation from Alltech

Study design, samplings, and chemical analyses:

In the experimental design, farm/herd was the experimental unit. Each product was fed to eight Danish Holstein herds in a cross over study with herds organized in dairy farm clusters. Cluster 1 was located in the northern part of Jutland (experimental platform maintained in collaboration with LandboNord, Brønderslev, Denmark) and Cluster 3 in the southern part of Jutland (experimental platform maintained in collaboration with (Jysk Landbrugsrådgivning, Esbjerg, Denmark). Herds within cluster were blocked according to grass-clover silage cutting strategy. Within block one herd was randomly picked for treatment sequence (Control followed by Yeast) and the other herd allocated to treatment sequence (Yeast followed by Control). Detailed description of study design and farm characteristics are given in tables below.

The study design was blinded for farmers, local veterinarians, extension staff, and technicians involved in sampling and analysis of samples. Placebo product was labelled as product with permission from the Danish Veterinary and Food Administration (FVST 2012-11-06). Bags with product were labeled by treatment codes:

- AV (placebo/control) and BV (treatment with live yeast) for Actisaf.
- I (treatment with autoclaved yeast) and II (placebo/control) for Levabon Rumen.

The experimental periods were 6 weeks.

Milk recording, efficiency control including sampling of PMR/TMR rations, as well as feces and urine sampling from 15 cows approximately 100 days after calving were done in the last treatment week in each of the two periods.

Farms involved in the study were typical of the Danish dairy production system with both grass-clover and corn silage in the rations. All farms fed silages of grass-clover mixes and corn grown and ensiled on farm. All farms supplemented the diets with commonly used commodities, such as canola meal and soybean meal as well as various small grain products and mineral premixes. Three out of eight farms in the northern cluster used potato products in the diets (0.1 to 2.2 kg DM/d). Six out of eight farms in the southern cluster used automatic milking, while no farms in the northern cluster used automatic milking.

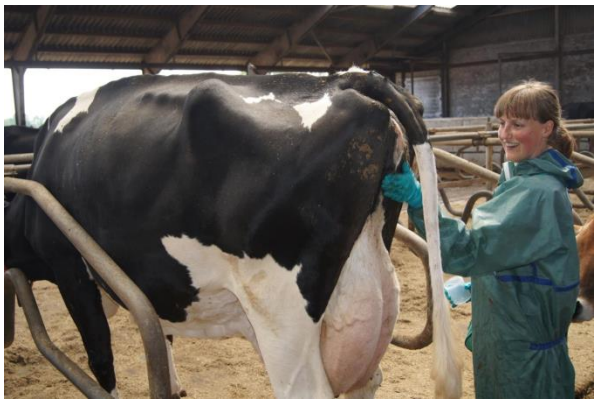
Efficiency control data were based on DMS-NorFor calculations (NorFor server version 1.20.3.1).

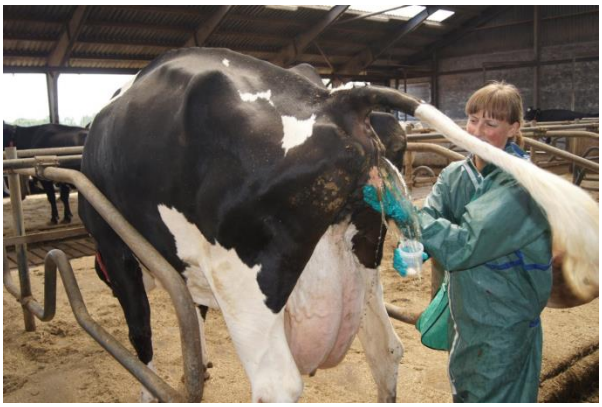
Feed samples were collected during feed out in 60-L barrels, and sample reduction was done by quartering.

Sampling of TMR/PMR during feed out

Sample reduction by quartering

Fecal samples were collected by rectal collection and mid-stream urine samples obtained after stimulation of the cows.





Sampling of mid-stream urine by manual stimulation of cows.

Samples of feed, feces, and urine were kept cool during transport and laboratory handling was initiated within 24 hours of collection.

Milk data were obtained from the Danish milk testing laboratory (Eurofins Steins A/S, Holstebro, Denmark) and milk yield quantified using standard procedures of the Danish milk-testing organization (RYK, Aarhus, Denmark).

Feed samples were analyzed for DM [NorFor 60°C DM corrected for volatile loss (residue * 0.99) +10], starch (enzymatic), NDF (amylase treated and ash corrected), crude protein (DUMAS), and in vitro digestibility (Tilley and Terry, 1963). Yeast products and placebo products were analyzed for chemical composition using the same methods as used for feed samples. Yeast products were also analyzed by ICP for concentrations of elements and yeast, molds (NMKL method 98) and aerobic microorganisms (NMKL method 86).

Fecal samples were dried at 60°C and ash determined by oxidation at 550°C. Fecal NDF and fecal indigestible NDF were determined by Ankom Neutral Detergent Fiber in Feeds Filter Bag Technique (Ankom Method 6 4/13/11) and the fecal indigestible NDF fraction was determined by Ankom In Vitro True Digestibility using the DAISY^{II} Incubator (ANKOM Technology Method 3).

Urinary pH was determined by glass electrode and urine samples were scanned by FT-IR using MilkoScan FT-120 (FOSS A/S, Hillerød, Denmark). Data on urine variables are based on PLS models on FT-IR spectra calibrated against the following methods: Urea determined using the monoxime diacetyl method (Marsh et al., 1965) using a continuous flow analyzer (Autoanalyzer 3, Seal Analytical Ltd., Burgess Hill, UK). D-3-Hydroxybutyrate (BHBA) determined using a Cobas Mira autoanalyzer (Triolab A/S, Brøndby, Denmark) and a kit D-3-hydroxybutyrate dehydrogenase (Ranbut, Randox Laboratories Ltd.). Creatinine was determined on 1:20 dilution of urine determined using a Cobas Mira autoanalyzer and a kit based on reaction with alcaline picrate (Creatinine 120 CP; Horiba ABX, Montpellier, France). Urine concentrations of allantoin and uric acid were determined by HPLC according to Thode (1999).

Calculations and statistical analysis:

Diuresis was calculated based on a total daily excretion of 114 mmol creatinine per cow (Røjen et al., 2011). Creatinine correction of urinary compound concentration was calculated as urinary concentration of compound / urinary concentration of creatinine.

Farm was the experimental unit and statistical analyses were done on datasets with 1 observation for each farm x period combination. For data with more than one observation per period (e.g. milk recordings) means were computed prior to statistical analysis.

Milk production based on test-day data was based on cows from 1 to 305 days in milk. To adjust for changes in average days in milk or parity within herd the ECM yield relative to predicted yield was computed for all cows 1 to 305 days in milk. The prediction model was based on herd record for the previous 4 years. The prediction model was based on the Wilmink model (Wilmink, 1987) and assuming peak milk production at approximately 50 days in milk. Model parameters for ECM yield were estimated using the Mixed procedure in SAS (SAS 9.2) with parity, parity x parameter for increase towards peak, and parity x days in milk as fixed effects. Cow ID x parity and year x quarter were included as random effects. The model parameters were estimated for each herd by running the model by herd.

Effects of treatment were calculated for efficiency control data, test-day data as well as urine and feces data using the Mixed procedure of SAS. The model included the fixed effects of treatment sequence, period, treatment, cluster and the treatment by cluster interaction. Herd by sequence was designated as a random effect. The treatment by cluster interaction was used to assess differences between products, although it has to be considered that the interaction between product and cluster also included differences in the susceptibility between clusters to yeast supplementation.

Data are presented as means ± standard error of the mean, if not otherwise stated. Significance level was declared at $P \leq 0.05$ and tendencies were considered at $0.05 < P \leq 0.10$. Means within cluster were separated using the PDIFF option of the LSMEANS statement protected by the overall F-test.

Protocol for Cluster 1 (North; 'live' yeast)

North: ChemVet dk A/S. Product: 5 g Actisaf Sc47 (50 * 10^9 CFU/cow/d) plus 20 g carrier.

Experimental treatments:

Treatment: 25 grams product per cow per day added to PMR/TMR.
Control/placebo: 25 grams placebo product per cow per day added to PMR/TMR – carrier only.

Period 1

Farm	Block	Treatment in period 1	Start date	Sampling date
1	1	Yeast1	20121210	20130121
2	2	Control	20121210	20130121
3	2	Yeast1	20121210	20130121
4	1	Control	20121210	20130121
5	3	Yeast1	20121211	20130122
6	4	Control	20121211	20130122
7	3	Control	20121211	20130122
8	4	Yeast1	20121211	20130122

*

Period 2

Farm	Block	Treatment in period 2	Start date	Sampling date
1	1	Control	20130122	20130306
2	2	Yeast1	20130122	20130306
3	2	Control	20130122	20130306
4	1	Yeast1	20130122	20130306
5	3	Control	20130123	20130307
6	4	Yeast1	20130123	20130307
7	3	Yeast1	20130123	20130307
8	4	Control	20130123	20130307

Herds in trial

Farm	Block	No of cows	Production level (kg ECM)	Milking system	Barn	Mixer
1	1	190	9,600	Parlour	Free stall	Kuhn, 27
2	2	260	10,200	Parlour	Free stall	JF-Stoll, 32
3	2	266	8,900	Rotary	Free stall	Keenan, 20
4	1	194	9,800	Parlour	Free stall	Kuhn, 27
5	3	167	10,200	Parlour	Free stall	Keenan, 20
6	4	156	10,500	Parlour	Free stall	Keenan, 20
7	3	128	8,500	Parlour	Deep bedding	Farasin, 22
8	4	166	10,200	Parlour	Free stall	Trioliet, 20

Protocol for Cluster 3 (South; 'autolysed' yeast)

Product tested: Biomin Levabon Rumen E

Experimental treatments:

Treatment: 15 grams product per cow per day added to PMR/TMR.
Control/placebo: 15 grams placebo product per cow per day added to PMR/TMR.

Period 1 (note that actual start date was delayed 4 days)

Farm	Block	Treatment in period 1	Start date*	Sampling date
1	1	Control	20121217	20130128
2	2	Yeast1	20121217	20130128
3	3	Yeast1	20121217	20130128
4	1	Yeast1	20121217	20130128
5	3	Control	20121218	20130129
6	2	Control	20121218	20130129
7	4	Control	20121218	20130129
8	4	Yeast1	20121218	20130129

***Actual start date delayed with 4 days relative to plan.**

Period 2

Farm	Block	Treatment in period 2	Start date	Sampling date
1	1	Yeast1	20130129	20130313
2	2	Control	20130129	20130313
3	3	Control	20130129	20130313
4	1	Control	20130129	20130313
5	3	Yeast1	20130130	20130314
6	2	Yeast1	20130130	20130314
7	4	Yeast1	20130130	20130314
8	4	Control	20130130	20130314

Herds in trial

Farm	Block	No of cows	Production level (kg ECM)	Milking system	Barn	Mixer
1	1	163	9,800	AMS	Free stall	Peecon
2	2	144	10,000	AMS	Free stall	Redrock
3	3	273	8,900	AMS	Free stall	Cormall
4	1	128	9,900	AMS	Free stall	Keenan
5	3	208	10,200	AMS	Free stall	Kuhn
6	2	226	10,300	Parlour	Free stall	JF-Stoll
7	4	131	11,200	Parlour	Free stall	RMH
8	4	148	10,200	AMS	Free stall	JF-Stoll

RESULTS AND DISCUSSION

Chemical composition of yeast and placebo products

Table 1 shows the chemical and microbiological characteristics of test products and document that Actisaf BV contains live yeast and Biomin I only low CFU counts for yeast.

Table 1. Microbiological and chemical characteristics of test products

Item	Actisaf AV (control)	Actisaf BV (treatment, 'live' yeast)	Biomin I (treatment, autolyzed yeast)	Biomin II (control)
Aerobic microorganisms, Log10 CFU/g	4.0	> 8.4	5.8	6.0
Molds, Log10 CFU/g	2.3	< 2.0	< 2.0	2.0
Yeast, Log10 CFU/g	**2.7**	**8.7**	**3.1**	**2.0**
DM, g/kg	945	939	945	898
Ash, g/kg DM	706	550	66	49
CP, g/kg DM	47	124	417	166
Soluble CP, g sCP/kg CP	320	129	618	278
aNDF, g/kg DM	47	79	147	636
Ca, g/kg DM	135	111	4.0	2.6
P, g/kg DM	1.5	3.9	9.4	7.8
Mg, g/kg DM	26.8	17.3	1.6	3.3
K, g/kg DM	5.6	7.9	11.7	7.7
Na, g/kg DM	2.3	1.5	3.7	3.5
Cl, g/kg DM	2.6	1.0	1.1	0.8
Fe, g/kg DM	14.6	11.3	0.8	0.3
Mn, g/kg DM	0.4	0.2	0.04	0.3
Zn, g/kg DM	0.4	0.2	0.1	0.7
Cu, g/kg DM	0.01	0.01	0.01	0.04
Se, g/kg DM	0.003	0.002	<0.001	0.005

Efficiency control data

Energy corrected milk yield, dry matter intake (DMI), concentrate intake as well as total diet concentrations of NDF, starch, crude protein (CP), fatty acids, and CAB (Na + K – Cl – S) did not differ between Control and Yeast (Table 2). DMI was numerically less for Yeast compared with Control for both treatments. With Actisaf, concentrate intake was numerically less (0.4 kg/d) for Yeast compared with Control, although it was not possible to detect the difference (P = 0.16 for Yeast x Product). Multiple minor adjustments of diets caused the numerically lower concentrate intake with Yeast for Actisaf. To maintain the nutrient composition of diets most herds made small adjustments in diet formulation during the 12 week period of the experiment. Concentrate intake is given as NorFor concentrate meaning that all feedstuffs with theoretical particle size less than 6 mm are considered as concentrate. Adjustments, affecting amount of concentrate, were found to be related to diet adjustments involving: canola meal, pelleted sugar beet pulp, small grains, soybean meal, pelleted protein mix and pelleted concentrate (see also data presented in Figure 2).

Energy efficiency increased (P = 0.05) for Yeast compared with Control. No indication for difference between yeast products was observed (P = 0.75 for Yeast x Product). The overall treatment difference was 1.8 ± 0.8 %-units difference between Yeast and Control for energy efficiency, energy efficiency being greatest with Yeast.

Nitrogen efficiency (% of total N intake secreted in milk) tended (P = 0.08) to be affected by Yeast x Product in agreement with a numeric increase for Actisaf and a numeric decrease for Levabon Rumen.

Energy efficiency was calculated from milk production, estimated maintenance requierements, estimated requirements for gain and feed consumption. Therefore measured feed intake impact the calculated efficiency and the differences in feed intake between treatment periods were strongly correlated with the differences in energy efficiency (r = -0.68 P < 0.01; see Figure 1). Likewise milk production was expected and found to be positively correlated to treatment difference in energy efficiency (r = 0.59 P = 0.02). These correlations are strongly significant, despite inability to detect treatment differences for DMI, milk yield, or ECM yield.

Table 2. Efficiency control data

	Cluster 1 (North) Actisaf		Cluster 3 (South) Levabon Rumen			P-value	
Variable	**Ctrl**	**Yeast**	**Ctrl**	**Yeast**	**SEM**	**Yeast**	**Yeast x Product**
Efficiency control data							
EC_ECM yield, kg/d	31.0	30.7	32.2	32.2	0.9	0.63	0.71
DMI, kg/d	22.3	21.8	22.5	22.2	0.4	0.12	0.74
Concentrate (NorFor), kg DM/d	9.2	8.8	8.5	8.5	0.7	0.25	0.16
NDF, g/kg DM	323.5	325.4	319.8	315.6	6.9	0.82	0.55
Starch, g/kg DM	161.9	163.3	183.0	175.4	9.3	0.35	0.19
CP, g/kg DM	166.5	162.6	169.4	175.3	3.4	0.73	0.11
Fatty acids, g/kg DM	33.8	32.5	30.5	30.0	1.9	0.24	0.61
CAB, meq./kg DM	174	203	181	185	16	0.11	0.22
Energy efficiency[1], %	**96.3**	**98.3**	**99.9**	**101.4**	**2.1**	**0.05**	**0.75**
Nitrogen efficiency, %	28.2	29.1	29.7	29.2	0.9	0.63	0.08

[1]Energy efficiency calculated relative to NorFor estimation of energy requirement for maintenance, gain, and milk production for all cows.

Increased energy efficiency would be considered as a very positive effect of yeast supplementation, however. However it is a matter of concern that the observed energy efficiency appears as the result of numerically decreased feed intake and a less numerical decrease in milk production. Although treatment differences for milk yield and feed intake variables cannot be detected in independent tests, the correlations in the responses still identify these variables as dominating driving forces in the observed effect on energy efficiency.

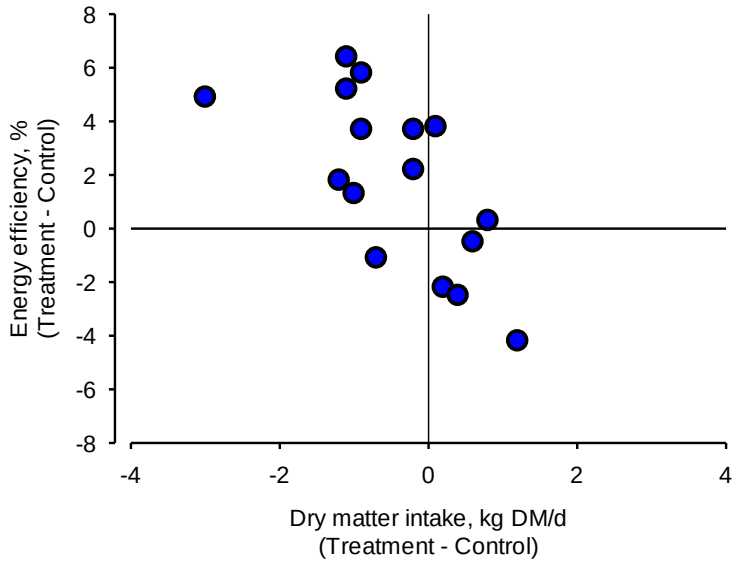


Figure 1. Figure shows the negative correlation between the calculated (treatment – control difference) for dry matter intake and calculated (treatment – control difference) for energy efficiency ($r = -0.68$ $P < 0.01$). Each data point represents the value from 1 farm/herd. The plot includes data from both clusters/products.

Danish dairy rations differ generally from rations in southern Europe and the US by relatively large contents of grass-clover silages based on perennial ryegrasses. To what extent this difference explains lack of feed intake response and lack of milk production response relative to the general published data on yeast (Desnoyers et al., 2009) is unknown. It has previously been found that the treatment response to yeast supplementation decreased with increasing NDF content of the diet and that the treatment response was affected by digestion kinetics of NDF (Robinson and Erasmus, 2009). The present study shows that cows fed a common Danish dairy ration do not respond to yeast supplementation with increasing feed intake and milk production.

Test-day data

Test-day milk recording was performed in the last week of each treatment period. Milk yield, ECM yield, and milk fat % were not affected by treatment ($P = 0.49$ to $P = 0.80$; Table 3). Milk protein % was affected by Yeast x Product interaction ($P = 0.03$) reflecting a greater reduction in milk protein concentration with Actisaf (≈ 0.07 %-units reduction) compared with Levabon Rumen (≈ 0.007 %-units reduction). Somatic cell counts and ECM yield relative to predicted yield by the Wilmink model and corrected for any drift in party or days in milk between treatments were not affected by treatment ($P = 0.33$ to $P = 0.50$).

Table 3. Test day data

	Cluster 1 (North) Actisaf		Cluster 3 (South) Levabon Rumen			P-value	
Variable	**Ctrl**	**Yeast**	**Ctrl**	**Yeast**	**SEM**	**Yeast**	**Yeast x Product**
Test-day data							
Milk yield, kg/d	31.3	31.6	35.0	34.9	0.9	0.74	0.53
ECM yield, kg/d	32.5	32.4	34.4	34.2	0.8	0.68	0.80
Milk fat, %	4.33	4.30	3.94	3.93	0.07	0.49	0.80
Milk protein, %	**3.49[a]**	**3.42[b]**	**3.43**	**3.43**	**0.03**	**0.01**	**0.03**
SCC, x 1000	250	259	260	225	41	0.55	0.33
Milk yield relative to prediction by Wilmink model	105.2	103.6	106.6	106.5	2.4	0.33	0.40

[a, b]Different superscripts within cluster indicate that means differ (P < 0.05).

One could speculate that the negative response in milk protein % to yeast supplementation was related to the numeric decrease in concentrate intake for Yeast compared with Control (see Table 1). However, the differences (Yeast period – Control period) for milk protein % and concentrate intake were not correlated (P = 0.28, see Figure 2) for Actisaf.

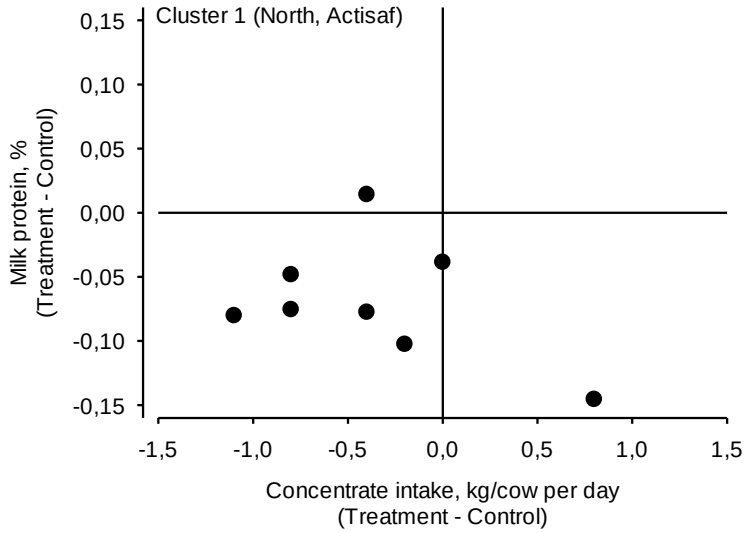


Figure 2. Plot of difference, Treatment - Control, for milk protein % and concentrate intake. Plot shows that the treatment difference in milk protein % was not correlated (P = 0.28) with difference in concentrate intake between treatment periods for the northern cluster supplemented with Actisaf. Each data point represents the value from 1 farm/herd.

For cluster 1/Actisaf, the treatment response in milk protein % was correlated (r = -0.83, P < 0.01) with the difference in total ration CP concentration between treatment periods (see Figure 3). However, this correlation cannot explain the overall negative treatment effect in milk protein % to Actisaf, as both positive and negative differences were associated with a negative response in milk protein % to Yeast.

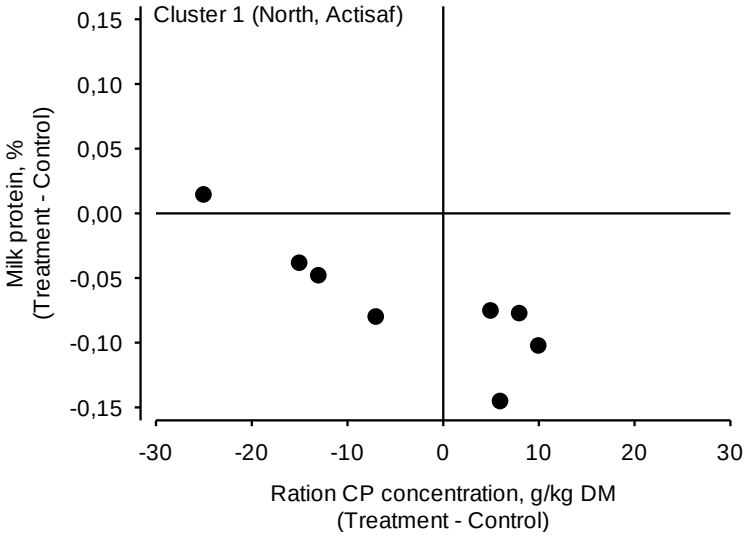


Figure 3. Plot of difference, Treatment – Control, for milk protein % and CP concentration of diets. The plot shows that the treatment difference in milk protein % was correlated (r = -0.83, P < 0.01) with the difference in dietary CP concentration between treatment periods. However, it is apparent that the correlation does not

indicate that the difference in CP between treatment periods explains the negative treatment effect on milk protein %. Each data point represents the value from 1 farm/herd.

For cluster 3/Levabon Rumen, no correlation between milk protein % and difference in total ration CP concentration between treatment periods was observed (P = 0.29).

In a literature summary, based on published studies investigating effects of yeast supplementation (Robinson, 2013), it was concluded that yeast products generally decreased milk protein %. Products differed in the extent of milk protein depression and in the proportion of trials detecting this effect with the 'dead yeast/autolysed yeast' were associated with less depression of milk protein concentration compared with 'live yeast'. The general findings of the literature review are in good agreement with the findings of the present study in which a negative impact on milk protein % was associated with Actisaf ('live yeast') and not Levabon Rumen ('autolysed yeast').

TMR/PMR composition

To ensure that efficiency control data were based on appropriate assumptions concerning the chemical composition of feed mixes fed to cows in the study, feed mixes were sampled at the time of efficiency control. The feed mixes were analyzed for composition of major nutrients and the difference between predicted and analyzed composition was calculated (Table 4). Dry matter concentration tended (P = 0.08) to be lower with Yeast compared with Control in the predicted feed mixes. This difference was not detectable in the analyzed composition (P = 0.53). The difference between predicted and analyzed DM concentration was not affected by treatment, however the greater numerically predicted difference in DM for Yeast compared with Control tend to over predict feed consumption in Control compared with Yeast. Recalculation of the statistics for feed efficiency using the difference between predicted and analyzed DM composition as a covariate reduced the treatment effect from significant (P = 0.05) to a tendency (P = 0.07). However, data still indicate that yeast supplementation increased energy efficiency in the present study.

In general the analyzed nutrient composition verified the values used in feed efficiency calculations. For CP, predicted values showed a greater numerical difference between treatments in Cluster 3 (Levabon Rumen) compared with analyzed composition. This difference explains the numerical trend for a lower N efficiency with Levabon Rumen. Based on the analyzed composition it appears that Levabon Rumen does not decrease N efficiency.

Table 4. Control of TMR/PMR composition connected to efficiency control

	Cluster 1 (North) Actisaf		Cluster 3 (South) Levabon Rumen			P-value	
Variable	**Ctrl**	**Yeast**	**Ctrl**	**Yeast**	**SEM**	**Yeast**	**Yeast x Product**
TMR/PMR predicted composition from efficiency control							
DM, g/kg (NorFor)	423.5	417.5	413.5	400.2	15.8	0.08	0.48
CP, g/kg DM	159.4	155.7	153.5	160.6	4.5	0.60	0.11
OMD, %	79.5	78.9	75.9	76.5	1.4	0.98	0.10
Starch, g/kg DM	153.2	150.7	192.6	185.7	10.2	0.20	0.54
NDF, g/kg DM	336.9	340.6	337.8	330.4	8.6	0.73	0.32
Analyzed composition of TMR/PMR							
DM, g/kg (NorFor)	402.9	403.9	409.8	399.3	13.2	0.53	0.45
OMD, %	75.7	75.0	76.3	76.0	0.7	0.41	0.71
Starch, g/kg DM	137.8	137.2	189.0	196.9	9.9	0.66	0.61
CP, g/kg DM	155.3	157.6	171.4	165.6	4.7	0.69	0.34
NDF, g/kg DM	336.6	336.3	333.8	333.6	10.0	0.97	0.99
Predicted – analyzed composition							
Difference_DM	20.6	13.6	3.7	1.0	8.9	0.59	0.81
Difference_OMD	3.8	4.0	-0.3	0.5	1.8	0.45	0.60
Difference_starch	15.5	13.5	3.6	-11.2	8.7	0.35	0.47
Difference_CP	4.1	-1.9	-17.9	-5.0	4.6	0.46	0.05
Difference_NDF	0.3	4.3	4.1	-3.2	8.7	0.85	0.52

Fecal variables

Fecal DM was not affected by treatment (P = 0.67) or differently affected by yeast product (P = 0.23) neither were any indications for changes in fecal scores observed (Table 5).

Fecal ash, fecal NDF, fecal indigestible NDF or digestibility of fecal NDF were not affected by treatment (P = 0.32 to 0.99). The present study does not indicate that yeast supplementation to rations common in Denmark alters fecal NDF content or fecal NDF characteristics.

Table 5. Fecal variables

	Cluster 1 (North) Actisaf		Cluster 3 (South) Levabon Rumen			P-value	
Variable	**Ctrl**	**Yeast**	**Ctrl**	**Yeast**	**SEM**	**Yeast**	**Yeast x Product**
Fecal dry matter, g/kg	123.0	121.2	122.8	126.5	0.4	0.67	0.23
Fecal score, 5 point scale	2.89	2.81	2.84	2.82			
Ash in feces, g/kg DM	147.2	140.9	133.0	133.0	4.7	0.33	0.32
NDF in feces (Ankom), g/kg DM	516.2	515.1	527.4	516.7	8.9	0.43	0.53
Indigestible NDF, g/kg DM	397.0	396.3	390.6	390.0	8.3	0.92	0.99
In vitro digestibility of fecal NDF (Ankom), %	22.90	22.89	25.79	24.37	1.31	0.36	0.37

Urinary variables

Yeast supplementation did not affect diuresis (P = 0.62; Table 6). Urea excretion in urine was affected by Yeast x Product interaction (P = 0.02) reflecting a decreased urea excretion with Actisaf and numerically increased excretion with Levabon Rumen. Predicted allantoin excretion was not affected. However, predicted uric acid excretion was affected by interaction between Yeast x Product (P = 0.05).

Urinary excretion of 3-hydroxybutyrate, urinary pH, and excretion of base equivalents in urine were not affected by treatment (P = 0.17 to P = 0.71).

Table 6. Urinary variables. All variables except urinary pH are based on FT-IR scans of urine samples and undisclosed PLS models

	Cluster 1 (North) Actisaf		Cluster 3 (South) Levabon Rumen			**P-value**	
Variable	**Ctrl**	**Yeast**	**Ctrl**	**Yeast**	**SEM**	**Yeast**	**Yeast x Product**
Diuresis, L/d (model)	19.2	18.2	20.8	20.6	1.6	0.62	0.76
Urea, creatinine corrected (model)	**35.8[a]**	**31.9[b]**	**40.7**	**43.4**	**2.3**	**0.65**	**0.02**
Allantoin, creatinine corrected (model)	0.49	0.44	0.46	0.45	0.03	0.12	0.38
Uric acid model, creatinine corrected (model)	**0.18[a]**	**0.15[b]**	**0.16**	**0.17**	**0.01**	**0.08**	**0.05**
3-hydroxybutyrate, creatinine corrected (model)	0.18	0.16	0.18	0.17	0.01	0.17	0.61
Urine pH	8.25	8.24	8.11	8.13	0.05	0.85	0.71
Titration of base equivalents, creatinine corrected (model)	20.9	22.7	17.8	17.7	2.5	0.64	0.62

[a, b]Different superscripts within cluster indicate that means differ (P <= 0.05).

It was evaluated if differences in ration composition explained the observed effects on urea excretion in the study, but none of the treatment – control differences for feeding variables correlated (P > 0.10) with the response in urea excretion. Therefore it appears that the urea excretion response is in fact a response to yeast supplementation and again points towards differences between ‘live yeast’ and ‘autolysed yeast’.

CONCLUSION

The present study investigated responses to yeast supplementation of dairy cow diets in 6-wk periods on variables related to milk production, nutrient efficiency, fecal NDF composition, as well as urinary markers for nutrition and physiological status. The study involved both ‘autolysed (dead)’ and ‘live’ yeast. Supplementation with yeast increased energy efficiency. However, the effect appeared as a result of numerically decreased feed intake in excess of a smaller numerically decreased milk production. It was not possible to detect effects on feed intake, milk yield, energy corrected milk yield, milk fat concentration or somatic cell count. Supplementation with ‘live’ yeast decreased milk protein concentration. Fecal NDF concentration and fecal NDF digestibility were not affected by yeast supplementation. Urinary variables for urea and uric acid excretion pointed towards opposite effects of ‘live’ and ‘autolysed’ yeast with urinary excretion reduced by ‘live’ yeast and increased by ‘autolysed’ yeast. Dairy cow diets commonly fed in Denmark differ from diets in countries, where studies often report positive production responses to yeast supplementation. The present study cannot demonstrate that yeast supplementation increases milk production or fiber utilization in dairy cows when supplemented to grass-clover and corn silage based rations which are common in Denmark.

IMPLICATIONS

Yeast supplementation did not affect feed intake, milk production and fecal variables according to the hypothesis (sales claims). However, yeast supplementation affected energy efficiency, milk protein concentration, and nitrogen metabolism variables. The mechanisms behind these effects are not well described and more work will be needed to explore the possibilities in yeast supplementation for affecting the biological efficiency of milk production.

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