

Identification of potential adverse effects of Bovaer feeding in practice

Advisory report from DCA – National Centre for Food and Agriculture, Aarhus University

Niels Bastian Kristensen, Maria Holst Kjeldsen & Morten Maigaard

Department of Animal and Veterinary Sciences, Aarhus University

Fact sheet

Title:	Identification of potential adverse effects of Bovaer feeding in practice
Authors:	Associate Professor Niels Bastian Kristensen, Postdoctoral Researcher Maria Holst Kjeldsen and Tenure-Track Assistant Professor Morten Maigaard, Department of Animal and Veterinary Sciences, AU
Peer review:	Senior Researcher Leslie Foldager, Department of Animal and Veterinary Sciences, AU Cabinet, AU, Senior Lecturer Peder Nørgaard, Department of Veterinary and Animal Sciences, University of Copenhagen
Quality Assurance, DCA:	Senior Consultant Johanna Höglund and Senior Consultant Louise Dybdahl Pedersen, DCA Central Unit, AU
Commissioning authority:	Danish Veterinary and Food Administration (now the Danish Food, Agriculture and Fisheries Agency), Ministry of Food, Agriculture and Fisheries
Date of order/delivery:	23 October 2025 / 1 May 2026
Reference number:	2025-0907939
Funding: based	The report has been prepared as part of the “Framework Agreement on Research-based Authority Services” entered into between the Ministry of the Environment, the Ministry of Food, Agriculture and Fisheries and Aarhus University under ID no. 5.11 in the “Service Agreement on Livestock Production 2025–2028”.
External comments:	Yes. The Danish Food, Agriculture and Fisheries Agency has had the opportunity comment on a draft of the response. No comments were made on the response.
External contributions:	Yes. SEGES Innovation P/S, Aarhus N, Denmark, had a consultant extracted data from the Cattle Database with the consent of herd owners. Viking Danmark, Aarhus N, Denmark, and MSD (Merck & Co., Inc., Rahway, NJ, USA) have granted access to Sense-Hub data via the SenseHub online client. There has been no further input or other influence on the responses from these parties. The report has undergone external peer review by Senior Lecturer Peder Nørgaard of the Department of Veterinary and Animal Sciences, University of Copenhagen.
Comments on the commission:	See the foreword.
Comments on the report:	The report was prepared within a very limited timeframe, which is why it has not been possible to provide a complete analysis of the data. The report presents findings which, at the time of publication, have not undergone external peer review or been published elsewhere. Should these findings be published at a later date in journals with external peer review, changes may therefore occur.
Cite as:	Kristensen NB, Kjeldsen MH, Maigaard M. 2026. Identification of potential adverse effects of Bovaer feeding in practice. Advisory report from DCA – National Centre for Food and Agriculture, Aarhus University. 55 pages. Delivered: 01.05.2026.

Advice from DCA:

Read more at <https://dca.au.dk/raadgivning/>

Foreword

On 23 October 2025, the Danish Veterinary and Food Administration (now the Danish Agency for Food, Agriculture and Fisheries) a report with the aim of identifying any negative effects of Bovaer allocation through a study based on data from a sample of Danish dairy herds that used Bovaer in 2025. Following a clarification of expectations, the commission was updated on 28 November 2025.

Commission text:

“The purpose of this project is to investigate whether the adverse effects observed following the initial use of Bovaer in practice have a causal link that can be demonstrated through biological samples. The deliverable is to be a report that details the possible causal links between observed adverse animal health effects and the feeding of Bovaer. The deliverable must include the analysis and results of the data processing. Data processing and data collection capable of determining whether the adverse effects observed at the start of Bovaer use can be assessed as having a causal link directly or indirectly related to Bovaer, or whether they have arisen from another cause.”

The following rationale for the commission is set out in the annex to the commission text:

“The introduction of Bovaer into dairy herds in 2025 may have affected a large number of dairy herds, and reports from the field indicate a decline in milk yield, changes in milk composition, reduced feed intake, symptoms of feed poisoning and paralysis in cows. Many herds are believed to have been taken by surprise by the impact on production following the introduction of Bovaer, which is feared to have led to varying degrees of deviation from the requirement to provide feed containing 60 mg 3-NOP/kg DM for at least 80 days.”

To fulfil the request, AU urgently launched a very short-term project (December 2025 to April 2026) to collect data from Danish dairy herds that used Bova devices in 2025. This report presents the data and observations gathered. It has not been possible, within the short project period, to carry out a detailed validation of all the data, and there remain a number of possible approaches to modelling and statistical analysis of the data which it has not been possible to explore in this report.

Furthermore, it has not been possible to include the following aspects in this report:

Under animal experimentation licence 2025-199767, the collection of blood samples from cows suspected of being affected by Bovaer was initiated. A call was issued for dead cows for post-mortem examination at Aarhus University and for information on slaughter cows from herds fed with Bovaer, in order to examine the rumen and abomasal epithelium from the cows. The calls resulted in very few responses and the collection of an insufficient dataset to carry out data analyses at this stage.

The collection of data on rumination time, feeding time and alarms based on activity monitors proved to be more extensive than expected, and this data is therefore not included.

An analysis of the potential effect of Bovaer on reproductive outcomes could not be prioritised in this report due to the project's timeframe and the presumed extent of reproductive problems in the herds, as indicated by interviews with participating herds.

It is expected that the above-mentioned aspects can be included in future studies.

Table of Contents

1. Background.....	6
2. Response.....	7
Selection of project hosts.....	7
Description of participating herds	7
Data collection	7
Experiences with the allocation of Bova	8
Response to feeding with Bova	8
3. Overall discussion.....	50
4. Conclusion.....	53
5. References	54

1. Background

From 2025, dairy farmers with more than 50 cows per year who are not covered by the organic regulations have been required to reduce methane emissions. Methane emissions could be reduced either by administering the feed additive Bovaer for at least 80 days in 2025 or by providing a feed ration with a high content of fatty acids throughout the year.

Bovaer contains the active methane-reducing substance 3-nitrooxypropanol (3-NOP). The substance inhibits methane formation in the rumen of cattle and is an approved feed additive in the EU based on a scientific assessment carried out by the European Food Safety Authority, EFSA (Bampidis et al., 2021).

In October 2025, a large number of cases of problems in dairy herds were reported, which were allegedly caused by feeding Bovaer. A questionnaire survey initiated by SEGES Innovation P/S received responses from 644 herds, of which approximately two-thirds reported a decline in feed intake and/or falling milk production when using Bovaer. In particular, herds that reported reduced feed intake in connection with the use of Bovaer reported an increased incidence of digestive and metabolic disorders (Nielsen et al., 2025).

A series of feeding trials involving Bovaer have been conducted at Aarhus University, which have shown that Bovaer can have a negative effect on feed intake and a minor negative effect on energy-corrected milk yield (ECM) in dairy cows. However, the trials at Aarhus University did not reveal any observations suggesting significant health problems associated with the administration of Bovaer (Lund et al., 2026).

The project behind this report has sought to determine whether feeding or production conditions can be identified across selected dairy herds that might explain or be linked to the seemingly large differences in experiences with the use of Bovaer between Danish dairy herds.

2. Response

Selection of project hosts

Between 1 December and 12 December 2025, the project team behind this report contacted dairy farmers across the country. Of the dairy farmers contacted, 76 agreed to take part in the project. Of the 76 commitments, one withdrew as they did not wish to sign the cooperation agreement, one withdrew their acceptance, and one did not wish to receive a visit in connection with the data collection. The project thus comprises a total of 73 dairy farmers spread across a wide geographical area, representing Jutland, Funen and a single participant on Zealand.

All participating herds were visited between 13 January and 26 March 2026 with the aim of interviewing project hosts to validate the details regarding the use of Bova devices in the herd, enquire about experiences with the use of Bova devices, and collect information to describe production conditions.

Description of participating herds

Herd sizes in the study ranged from just under 150 to more than 1,000 cows. On average, the herd size was 378 lactating cows, and together the herds represented approximately 27,650 lactating cows, corresponding to just over 5.5% of the milk-recorded cows in Denmark.

The study included 17 herds consisting mainly of Jersey cattle and 56 herds consisting mainly of large breeds.

The average milk production (average of herd yields) was 12,620 kg EKM per lactating cow. The range was from 9,688 to just over 15,000 kg EKM per lactating cow across Jersey and large breeds. The average milk yield for herds in the yield recording scheme for the 2024–2025 recording year was 11,834, so the sample had a slightly higher yield than the average Danish dairy herd, even though there were slightly more Jersey herds (23%) in the sample compared to the proportion of Jersey herds nationwide (approx. 12%).

Two of the participating herds fed Bovaer throughout 2025. All other herds were registered for 80 days of Bovaer feeding in 2025. The herd that fed Bovaer for the shortest period fed Bovaer for 29 days. Of the herds registered for 80 days of Bovaer feeding, Bovaer was administered for an average of 81 days in 2025. A large proportion (64%) of the participating herds began administering Bovaer around 1 October 2025 (47 out of 73 herds).

Of the participating herds, 45 maintained the Bovaer dosage at the planned level of 60 mg/kg dry matter (DM) throughout the administration period, 18 herds administered approximately 45 mg/kg DM and 10 herds administered approximately 30 mg/kg DM. In general, the lower doses were used in herds where there were concerns about significant adverse effects from administering Bovaer at the full dose of 60 mg/kg DM.

Data collection

In addition to interviews with project hosts, the project carried out data extraction from the cattle database owned by Landbrug & Fødevarer and operated by SEGES Innovation P/S. Data from the cattle database was supplied to the project as raw data extracted from tables in the database, containing information on herd ID, animal ID, calvings, milk quality, health records (treatments) and performance monitoring data. Subsequent data processing was carried out within the project.

Data on feed checks, herd size, paratuberculosis and dead cows were obtained by the project via the cattle programme DMS (SEGES Innovation P/S). For all herds, at least one

feed inspection around the time of the introduction of Bovaer into the herd, and information was sought on the feed and mineral mixes included in the feed inspections.

Data on the composition of feed and mineral mixtures included in the feed inspections were obtained from bulk feed suppliers.

The salmonella status of the flocks was obtained by consulting the CHR register (<https://chr.fvst.dk/>).

Experiences with the allocation of Bovaer

This study is not an analysis of how the subjectively assessed experiences with the allocation of Bovaer can be explained or related to production conditions, but will focus on the analysis of more objective data.

Questions were asked about experiences with feeding Bovaer to ensure that the sample can reasonably be compared with the issues that have been raised in the public domain. Similar to the questionnaire survey conducted by SEGES Innovation P/S (Nielsen et al., 2025), approximately two-thirds of the project hosts in this study reported varying degrees of negative experiences with the allocation of Bovaer:

- 47 farmers (64%) experienced varying degrees of negative effects
- 26 farmers (36%) highlighted a negative feed intake response
- 21 farmers (29%) highlighted a negative response in milk production
- 19 farmers (26%) highlighted an increase in the number of acutely inactive cows
- 18 farmers (25%) highlighted an increased number of alerts from activity sensors
- 10 farmers (14%) highlighted a general reduction in cow activity
- 10 farmers (14%) highlighted an increased incidence of diarrhoea in the cows
- 2 farmers (3%) expressed a suspected impact on the herd's reproductive performance

The statements from the project hosts reflect their analysis of the period during which Bovaer was fed to the herd, and the effects highlighted may not necessarily have been present throughout the entire period. However, they likely provide a good picture of the concerns regarding the use of Bovaer in many herds and broadly align with the questionnaire survey conducted by SEGES Innovation P/S (Nielsen et al., 2025).

It is concluded that the sample of herds included in this study shares the experiences and concerns regarding the use of Bovaer that were evident in the public debate in autumn 2025.

Responses to feeding with Bovaer

Descriptive variables

To analyse differences in response to Bovaer feeding between herds, a number of descriptive variables have been collected, as shown in Table 1. No information is available regarding which feeding, nutritional or production factors may influence the cows' response to Bovaer. The present study focuses on the inclusion of a wide range of feeding and production variables, including breed, Bovaer allocation, feed composition, the nutrient composition of the feed, physical conditions related to feeding, some general housing conditions, and some general variables concerning herd health and performance levels.

Table 1. Variables used to analyse the response to feeding with Bovaer in the participating herds, stating the variable name, description and data source.

Descriptive variable	Description	Data source
Breed variable	The herd is described as either Jersey or a large breed. Categorical variable with 2 levels.	Key figures in DMS
Cows Product category	Describes how Bovaer is incorporated into the herd's diet: FarmPack (a mixture of oestrogen pellets, chalk and/or salt), minerals (in a mineral mix) or a raw material/concentrate mix. A categorical variable with 3 levels.	Package inserts and interviews with project hosts
Bovaer Weighing category	Describes how Bovaer is weighed into the feed mixer. Contains the same categories as the product category + weighing via home-mixed premix. Categorical variable with 4 levels.	Insert sheets, feed plans and interviews with project hosts
Bovaer Allocation category	Describes whether Bovaer is allocated in a base mix (PMR), supplemented with concentrate without Bovaer, or in a total mixed ration (TMR), which represents the entire feed ration. In one participating herd, Bovaer was added to both concentrate and PMR; this herd is categorised as TMR. Categorical variable with 2 levels.	Feeding plans and interviews with project hosts
Silage change upon introduction of Bovaer	Describes whether, at the same time as the introduction of Bovaer into the feed, there has been a significant replacement of silage batches, e.g. recently harvested maize silage as a substitute for the previous year's maize silage. Categorical variable with 2 levels.	Feeding plans and interviews with project hosts
Bovaer initiatives	Describes whether any deliberate changes have been made to the feeding regime, apart from a change in silage, in connection with the introduction of Bovaer, e.g. the addition of a toxin binder. A categorical variable with 2 levels.	Interview with project hosts
Bovaer dose variable	Describes the average estimated dose of Bovaer during the period of Bovaer administration relative to the normal dose of 60 mg 3-NOP/kg DM. Numerical variable with a value of either 50, 75 or 100 (i.e. 30, 45 or 60 mg/kg DM).	Interview with project hosts
Grass proportion of the ration	Proportion of ration dry matter consisting of grass silage. Numerical variable (proportion of ration dry matter).	Feed checks* in DMS

Maize proportion of the ration	Proportion of ration dry matter consisting of maize silage. Numerical variable (proportion of ration dry matter).	Feed check* in DMS
Proportion of rapeseed meal in the ration	Proportion of ration dry matter consisting of rapeseed meal. Numerical variable (proportion of ration dry matter).	Feed controls* in DMS and information from delivery notes and product cards
Proportion of total rapeseed in the ration	Proportion of ration dry matter consisting of rapeseed cake + rapeseed meal + rapeseed. Numerical variable (proportion of ration dry matter).	Feed checks* in DMS and information from delivery notes and product cards
Proportion of straw feed in the ration	Proportion of ration dry matter consisting of straw feed such as straw and hay. Numerical variable (proportion of ration dry matter).	Feed checks* in DMS
Proportion of cereal products in the ration	Proportion of ration dry matter consisting of cereals and maize (grain) – the grain content in maize and cob maize silage is not included. Numerical variable (proportion of ration dry matter).	Feed controls* in DMS and information from delivery notes and product cards
White clover content	White clover proportion in ration dry matter, expressed as the product of the estimated white clover content in grass silage on the farm and the grass proportion in the ration. Numerical variable (proportion of ration dry matter).	Feed checks* and interviews with project hosts
Water	Cows' water supply via own waterworks or from shared/public waterworks. Categorical variable with 2 levels.	Interview with project hosts
Feed mixing score	Describes the assessed intensity of feed mixing on the farm. Categorical variable with 2 levels.	Interview with project hosts and inspection of the premises
Feed residue	Describes whether the aim is for the cows not to be fed until the feed table is completely empty of feed, or whether the aim is to leave a feed residue that is removed before the next feeding. Categorical variable with 2 levels.	Interview with project hosts
Automatic milking	Describes whether automatic milking is used. Categorical variable with 2 levels.	Interview with project hosts and inspection of physical conditions
Partition	The type of partition separating the cows' walking area and feeding table. Categorical variable with 4 categories (pipes, feed barriers, 'flex-feed' or mixed).	Inspection of physical conditions
Rear edge of the feed trough	Describes the height and degree of separation between the cows' walking area and the feed on the feeding table. Categorical variable with 3 categories (low, medium, high).	Inspection of physical conditions

Light	Description of the use of light sources in the barn, particularly during the winter months. Categorical variable with 3 categories (low, medium, high light intensity).	Interview with project hosts and inspection of physical conditions
Bedding	Description of the cows' housing and bedding. Categorical variable with 7 categories (sand, straw-bedded mattress, water mattresses, manure fibres, straw-lime-water, deep litter or mixed).	Interview with project hosts and inspection of physical conditions
Yeast	Describes whether a yeast product has been added to the feed. Categorical variable with 2 categories (yes/no).	Interviews with project hosts and information from package leaflets and product cards
Energy concentration	Energy concentration of the feed ration MJ NEL/kg DM. Numerical variable.	Feed checks* in DMS
Roughage proportion	The proportion of roughage in the feed ration. Numerical variable (proportion of ration dry matter).	Feed controls* in DMS
Nutrient composition of the feed ration	The concentration of crude protein, fatty acids, NDF (fibre), starch, sugar, calcium, phosphorus, magnesium, potassium, sodium, chloride, sulphur, copper, cobalt and selenium. All numerical variables (g/kg DM, except mg/kg DM for copper, cobalt and selenium).	Feed checks* in DMS
Energy utilisation	The calculated energy utilisation. Numerical variable ($100 \times \text{NEL in feed intake} / \text{NEL in feed}$).	Feed controls* in DMS
Herd size	The number of yearling cows in the herd.	Key figures in DMS
Yield level within breed	Yield level within each breed in the herd. Numerical variable (yield in kg EKM/lactating cow).	Key figures in DMS
Herd cow age	Number of lactation years per cow to describe the herd's cow age. Numerical variable (years).	Key figures in DMS
ParaTB	The proportion of cows in the herd infected with paratuberculosis. Numerical variable.	Key figure in DMS
Salmonella	The herd's salmonella status (<i>Salmonella Dublin</i>). Categorical variable with 2 categories.	CHR register

*Feed controls are a model of the herd's feed conversion based on a complete record of feed allocated minus feed residues and the use of the NorFor feed evaluation system (<https://www.norfor.info/>).

Analyses of milk production response have been carried out on two different datasets. One dataset originates from milk recording, which is carried out 11 times a year for most herds; a few herds have 6 milk recordings per year. During the milk yield recording, milk production and milk composition are determined for each lactating cow in the herd. The second dataset contains data on milk composition, with data from each individual milk collection in the herds. This dataset includes an observation from every other day in most herds. Across herds,

for both datasets, the Bovaer effect on milk composition has been analysed by calculating the difference between the expected level at a given time of year when Bovaer is allocated and the expected level in the same period without Bovaer allocation.

Response analysis – yield control data – milk production

To analyse the milk production response to feeding with Bovaer, yield data for each herd in the years 2022, 2023 and 2024 were first used to parameterise a lactation model based on Wilmink (1987). The model describes a lactation number-specific (1st, 2nd or 3rd+ – where 3rd+ refers to all with lactation number 3 and higher) expected yield based on a non-linear function of days in lactation, which initially increases and, after a peak, slowly decreases.

The model describes expected yield as lactation number \times stage of lactation + lactation number \times $\exp(-0.05 \times$ stage of lactation). Stage of lactation is described in 20-day intervals. In the model, animal ID within lactation number and quarter within year are described as random effects. The model was optimised for each herd using PROC MIXED in SAS (SAS Institute Inc., Cary, NC, USA).

Calculation of ECM yield = kg milk \times $((0.383 \times$ fat percentage + $0.242 \times$ protein percentage + $0.7832)/3.14)$ based on Sjaunja et al. (1991).

The yield in kg EKM/day achieved in 2025 was predicted using the herd-optimised model for expected EKM yield (based on yields from the years 2022–2024, see above) for all cows that had been milk yield-monitored from calving until 305 days into lactation, i.e. using separate parameters for each herd. For each cow on each recording day, the actual EKM yield was divided by the predicted EKM yield, and the ratio was multiplied by 100 – thereby obtaining a relative yield in relation to the predicted yield.

Milk production generally varies with the seasons. Figure 1 shows the average yield relative to the predicted yield for all cows in the study during the periods when no Bova units were allocated. It should be noted that the average yield is higher than in previous years (relative yield is greater than 100%), and that there is an expected increase in yield in the spring, peaking in May, after which yield declines until November. The average relative yield shown in Figure 1 is used as a reference for the relative yield during the periods when the herds are fed Bovaer. The Bovaer effect is calculated as the difference between the relative yield achieved during a period of feeding with Bovaer minus the average value for herds not feeding with Bovaer during the same period (i.e. for the EKM yield values shown in Figure 1).

For each herd and lactation number (1st, 2nd and 3rd+), the difference was calculated between the relative yield achieved with Bova (relative yield is $100 \times$ actual yield in 2025 divided by predicted yield) and the seasonally adjusted reference yield (Figure 1). The seasonally adjusted reference yield was calculated for all participating herds that did not use Bovaer during the period in question. The differences in relative yields achieved during periods when Bovaer was administered are shown for first-calf heifers, as an example, in Figure 2. Figure 2 shows that there are herds with higher-than-expected yields and herds with lower-than-expected yields during the period when Bovaer is fed. It is also noted that a relatively large proportion of the participating herds begin Bovaer allocation in October 2025. It should be noted that both positive and negative deviations between expected and achieved yield occur in herds that started feeding Bovaer early in the year and in herds that started feeding Bovaer late in the year.

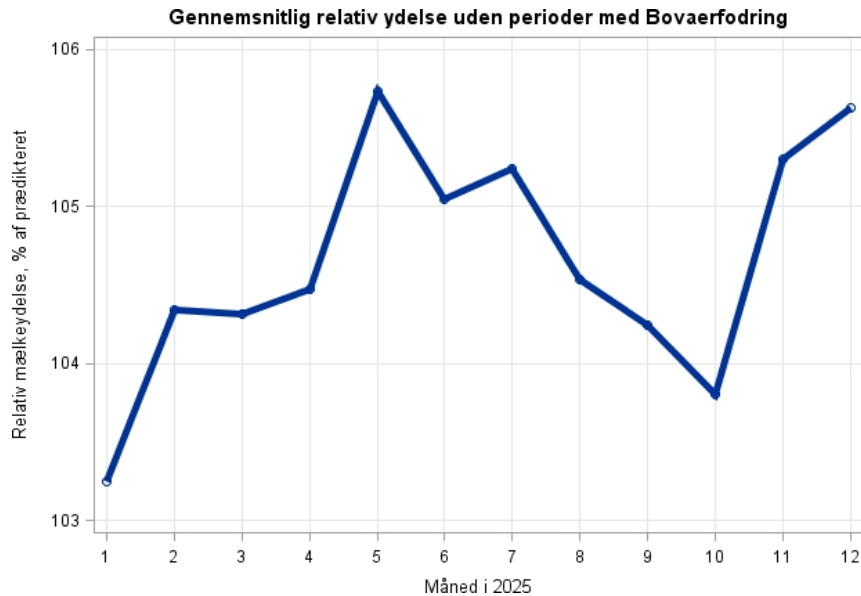


Figure 1. EKM yield relative to predicted yield for data obtained from yield checks where the herds did not feed Bovaer. The figure shows an expected seasonal variation in EKM yield when the yield is adjusted for lactation number, day in lactation and differences between herds.

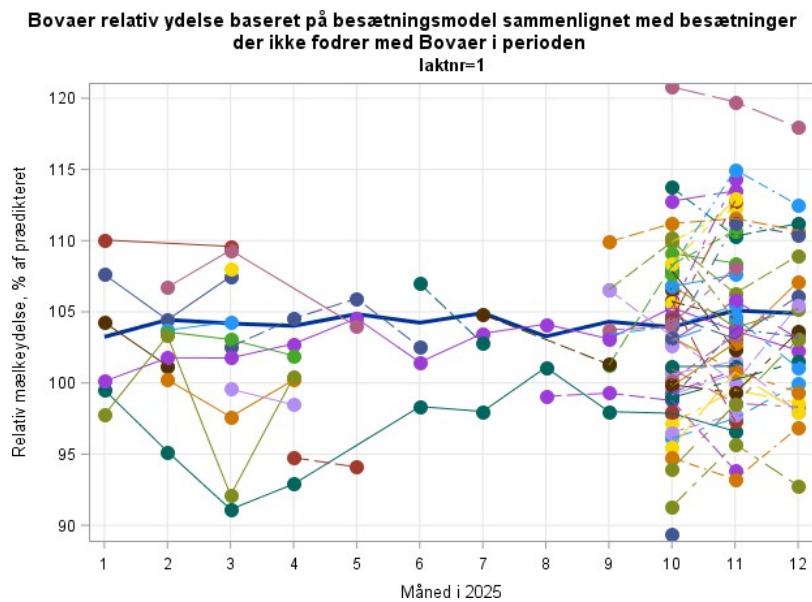
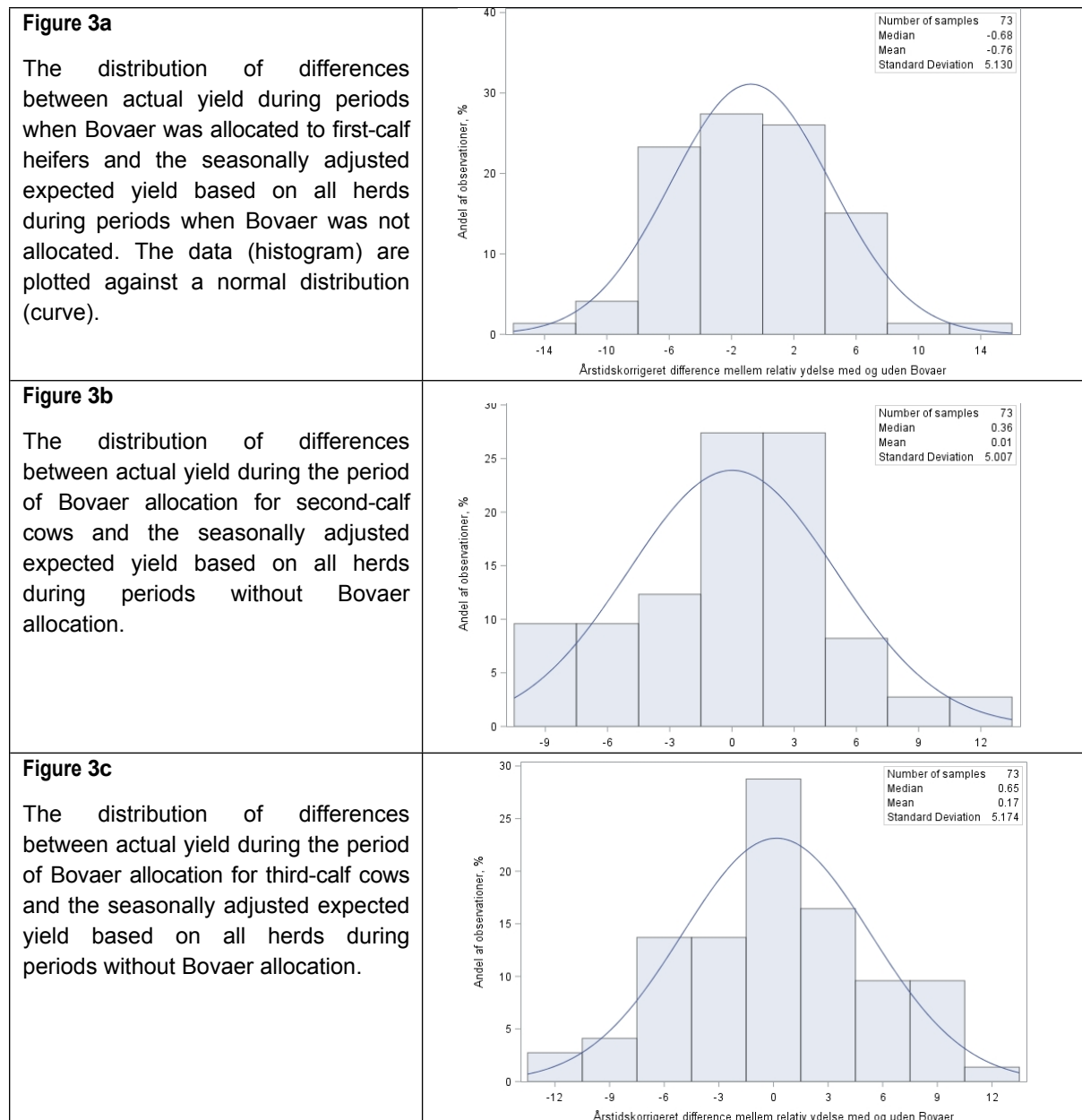


Figure 2. EKM yield for first-calf heifers in each of the 73 herds (coloured points) that fed Bovaer in 2025, as well as the expected seasonally adjusted relative EKM yield (the thick blue line) in 2025. Connected points show data from the same herd. The figure shows that some herds have higher yields than expected (the points lie above the thick line), whilst other herds have lower yields than expected (the points lie below the thick blue line).

Figures 3a, 3b and 3c show the distribution of the differences between the actual relative EKM yield during periods with Bovaer and the seasonally adjusted expectation during periods without Bovaer for first, second and 3+ calves. The average difference between the actual yield and the seasonally adjusted

expectation (\pm standard error of the mean, SEM) is -0.8 ± 0.6 , 0.0 ± 0.6 and 0.2 ± 0.6 percentage points for first-, second- and third-calf-plus cows, respectively. None of the mean differences differ from 0 ($P = 0.21$, $P = 0.99$ and $P = 0.79$).



Figures 3a, b and c. Effect of Bovaer allocation on EKM yield from the yield control, calculated as differences between relative yield based on a herd-specific model and a seasonally adjusted expected yield. Data are shown for cows in first lactation, second lactation and third+ lactation.

As the overall effect of Bovaer on EKM yield in the herds studied was not significantly different from 0, there was no general effect of Bovaer on EKM yield.

To investigate whether there were significant differences in how Bovaer affects individual herds, data were analysed using stepwise regression analysis via PROC GLMSELECT in SAS. To minimise the risk of over-parameterisation, Schwarz's Bayesian Information Criterion (Schwarz

Bayesian Information Criterion, SBC) was used for model selection, in addition to the requirement for significance of the included parameters. In all of these studies, a significance level of $P < 0.05$ was selected.

An average was calculated for the difference. The calculation was first performed within lactation numbers (1st, 2nd and 3rd+), then the average was calculated across lactations for each herd. The analysis of the average herd deviations was carried out taking into account all descriptive variables presented in Table 1.

The optimised model (Table 2) explains very little of the variation in the dataset (13%). The model includes a positive effect of magnesium and a negative effect of cobalt in the feed ration. Figure 4 shows the predicted versus observed values using the model. The result should be interpreted with great caution, as the modelling is based on residuals from a model without a main effect of Bovaer.

Table 2. Output from GLMSELECT analysis of EKM yield. The analysis was performed on a dataset with the average of all lactation groups within the herd.

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Value
Model	2	202.10746	101.05373	5.08
Error	70	1392.98288	19.89976	
Corrected Total	72	1595.09035		
Model Fit Statistics				
Measure	Value			
Root MSE	4.46091			
Dependent Mean	-0.19727			
R-Square	0.1267			
Adj R-Sq	0.1018			
AIC	296.25826			
AICC	296.84649			
SBC	228.12964			
Parameter Estimates				
Parameter	DF	Estimate	Standard Error	t-value
Intercept	1	-11.306754	3.940408	-2.87
Mg_gkgDM	1	4.530614	1.438618	3.15
Co_mgkgDM	1	-9.410610	4.197730	-2.24

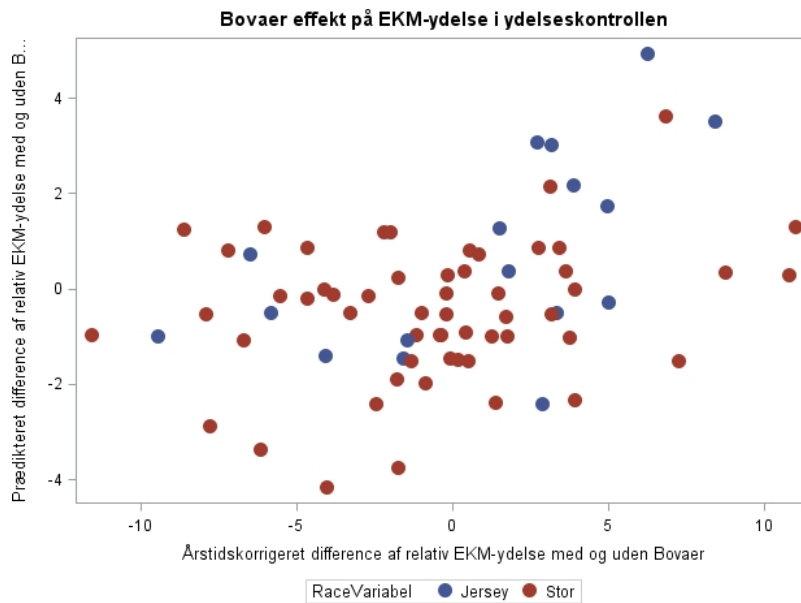


Figure 4. Predicted effect of Bovaer on EKM yield (based on the model shown in Table 2) plotted against observed differences in yield during the period of feeding with Bovaer, compared with expected relative yield based on a herd-specific model. The model has a very low coefficient of determination, and only 13% of the variation in the dataset is explained.

To test for underlying factors across the many descriptive variables in the dataset, a PLS (partial least squares) analysis was performed using PROC PLS in SAS. There were no significant underlying factors to describe the yield response to Bovaer, as Factor-1 in the PLS model explained only 8% of the variation in the observed yield difference.

In summary, it can be concluded that the EKM yield determined by the yield recording in the participating herds, which is a weighted average of milk volume and milk constituents, was not influenced by the administration of Bovaer. Significant effects of magnesium and cobalt in the feed rations on the difference in response to Bovaer between herds were found, but the model based on these variables explained only 13% of the variation between herds.

Response analysis – performance recording data – milk production – early lactation

To investigate whether cows early in lactation were particularly affected by Bovaer, an analysis was carried out using data representing only the period from 10 to 60 days into lactation, corresponding to the analysis described above for EKM yield over the entire lactation.

The effect of Bovaer on milk yield in early lactation was numerically very small and not significantly different from 0 for first-calf heifers (0.6 ± 0.8 percentage points, $P = 0.41$), second-calf heifers (0.2 ± 0.7 percentage points, $P = 0.75$) or 3. + calving cows (-0.2 ± 0.7 , $P = 0.76$).

The use of PROC GLMSELECT and SBC yielded an optimum with no parameters above the mean, i.e. none of the explanatory variables reduced the SBC in the model, meaning that no variable could contribute significantly to explaining the variation in the differences between the seasonally adjusted expected EKM yield and the actual yield achieved for cows early in lactation (data not shown).

A PLS analysis was also unable to identify underlying factors with a significant explanatory power for EKM yield in early lactation.

It can therefore be concluded that, based on the available data, EKM yield in early lactation could not be shown to be influenced by the allocation of Bovaer. Furthermore, none of the descriptive variables included contributed to explaining the differences between herds in yield during periods with

Bova administration compared with the seasonally adjusted reference based on herds that did not administer Bova.

Response analysis – yield control data – fat percentage

Data on milk fat percentage from the yield recording scheme were modelled as described above for EKM yield, based on Wilmink (1987). Figure 5 shows the seasonal variation in milk fat percentage over the period 2025, based on periods when no Bova sensors were in use in the participating herds. It should be noted that the relative seasonal effect on fat percentage was significantly greater than the seasonal effect on EKM yield, and that the lowest fat percentages were observed in the summer months, as well as a sharp increase in fat percentages from September to November.

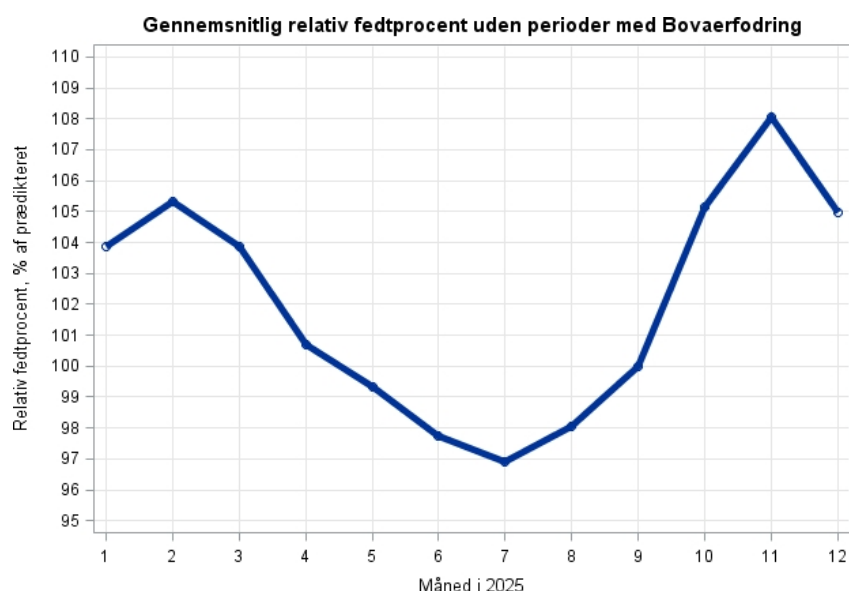


Figure 5. Fat percentage in milk samples from the milk recording scheme relative to predicted fat percentage. Data are based on periods without Bovaer allocation. The figure shows an expected seasonal variation in fat percentage when the fat percentage is corrected for lactation number, day in lactation and differences between herds. The figure shows that the fat percentage was high in 2025 compared to previous years.

The distributions of differences in fat percentage during periods with Bovaer allocation compared with periods without Bovaer allocation resulted in normally distributed differences corresponding to the distributions for EKM yield shown in Figure 3a–c. None of the differences were significant for fat percentage: 1st calf (-0.1 ± 0.6 , $P = 0.84$), 2nd calf (0.4 ± 0.6 , $P = 0.46$) and 3rd+ calf (0.4 ± 0.6 , $P = 0.49$).

To investigate which variables might explain differences in fat response to Bovaer between herds, PROC GLMSELECT and SBC were applied to the dataset containing average deviations (across lactation numbers) within herds. This yielded an optimal model with a number of significant parameters (Table 3). The model explained 55% of the variation in the dataset and includes effects of Bovaer product category, Bovaer administration category, Bovaer dosage, intervention, breed, potassium concentration in the feed, ParaTB status and salmonella status. Figure 6 shows the predicted versus observed values using the model.

Table 3. Results from a GLMSELECT analysis of differences between relative fat percentage during periods when Bovaer was administered and the seasonally adjusted reference for fat percentage in milk samples from the milk recording scheme during periods when Bovaer was not administered. The analysis was performed on a dataset comprising the average of all lactation groups within the herd.

Analysis of Variance				
Source	DF	Sum of	Mean Square	F
Model	9	707.26888	78.58543	8.69
Error	63	569.58572	9.04104	
Corrected Total	72	1276.85459		
Model Fit Statistics				
Statistic	Value			
Root MSE	3.00683			
Dependent Mean	0.22771			
R-Square	0.5539			
Adj R-Sq	0.4902			
AIC	244.97484			
AICC	249.30271			
SBC	192.87943			
Parameter Estimates				
Parameter	DF	Estimate	Standard Er-	t Value
Intercept	1	1.937256	5.847205	0.33
Bovaer Product Category Bovaer Pack	1	7.937324	3.221534	2.46
BovaerProductCategory Minerals	1	6.581925	3.198020	2.06
BovaerProductCategory Raw Material Mix-	0	0	.	.
BovaerAllocationCategory PMR	1	2.398381	0.775593	3.09
Bovaer Allocation Category TMR	0	0	.	.
BovaerDoseScore	1	0.066682	0.020850	3.20
Intervention Yes	1	4.222258	1.088400	3.88
Action No	0	0	.	.
RaceVariable Jersey	1	-2.502337	0.971981	-2.57
RaceVariable Large	0	0	.	.
K_kgDM	1	-0.855566	0.257934	-3.32
ParaTB_positive_proce	1	-1.581319	0.418657	-3.78
SalmonellaStatus 1	1	-3.992027	1.142683	-3.49
SalmonellaStatus 2	0	0	.	.

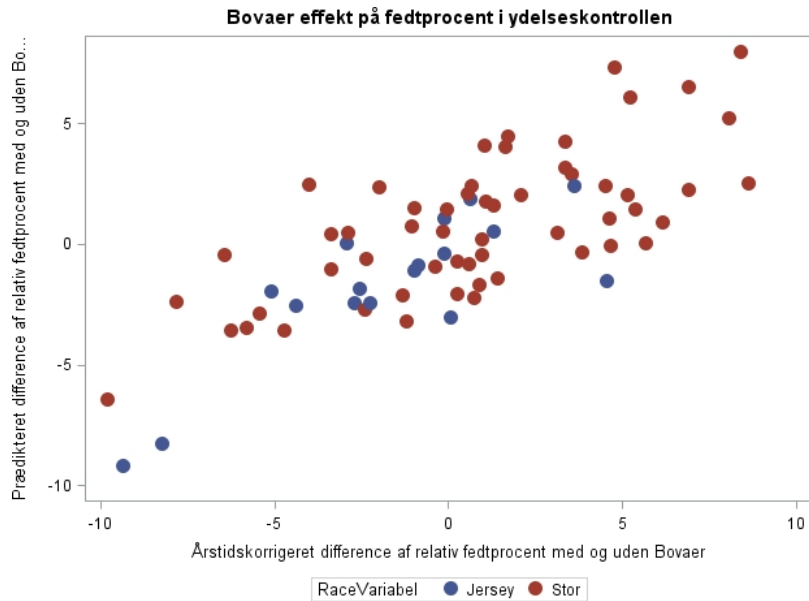


Figure 6. Predicted effect of Bovaer on differences in relative fat percentages from the performance test, based on the model presented in Table 3. The model explains 55% of the variation in the dataset.

Feeding Bovaer had no effect on the fat percentage in milk samples from the yield tests, but the variation between herds could largely be explained by the descriptive variables included in the statistical model. The result should be interpreted with great caution, as the modelling is based on residuals from a model without a main effect of Bovaer; however, as will be presented below, an effect of Bovaer on the fat percentage in milk delivered to the dairy was detected. It is therefore possible that in this modelling of fat percentage in yield control data, there is an underlying effect that simply could not be detected as a main effect of Bovaer.

Response analysis – yield recording data – protein percentage

Data for protein percentage from the yield control were modelled as described above for EKM yield based on Wilmink (1987). Figure 7 shows the seasonal variation in the relative protein percentage across 2025 based on periods without the administration of Bovaer in the participating herds. It should be noted that the relative seasonal effect for protein percentage shows the same pattern as that shown for fat percentage. The lowest protein percentages occur in the summer months and rise sharply from August to November.

The effect of Bovaer on the relative protein percentage, as the average of all lactations corrected for seasonal effect, was different from 0 (0.6 ± 0.3 , $P = 0.03$); see Figure 8.

Application of PROC GLMSELECT and SBC to the dataset for protein percentage, using within-herd average deviations (across lactation numbers), revealed effects of white clover (negative sign for parameter estimate), total rapeseed content in the ration (positive sign for parameter estimate), Jersey breed and calcium (Table 4). The model explained only 25% of the variation (Figure 9) in the dataset, and the variation in the Bovaer effect was therefore largely unexplained.

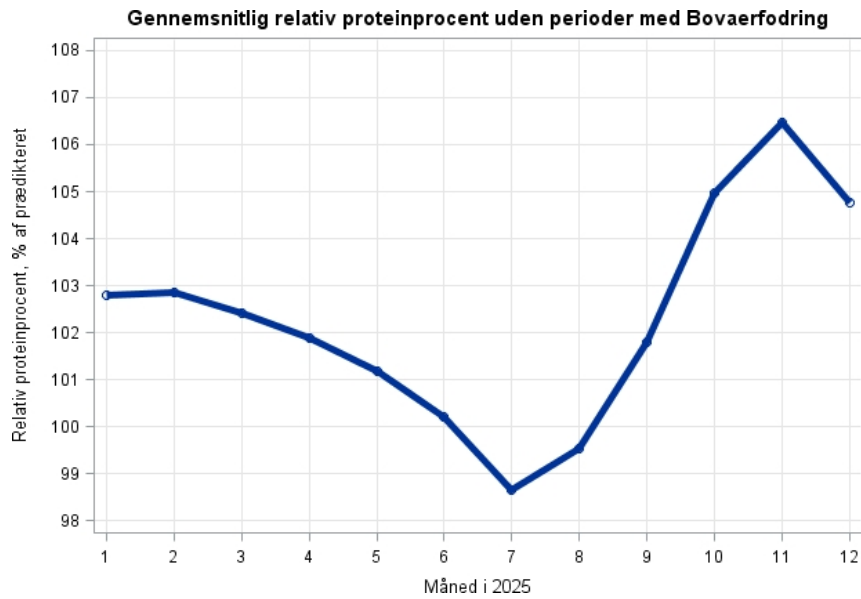


Figure 7. Relative protein percentage in milk samples from the yield checks based on predicted protein percentage in periods without Bovaer administration. The figure shows an expected seasonal variation in protein percentage when yield is corrected for lactation number, day in lactation and differences between herds. The figure shows that the protein percentage was high in the autumn and winter of 2025.

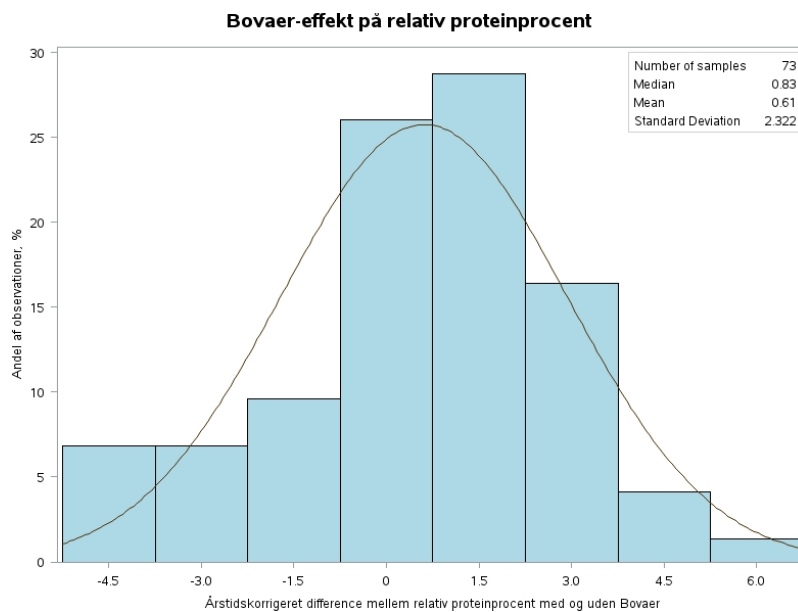


Figure 8. The distribution of differences between the actual protein percentage during periods when Bovaer was administered and the seasonally adjusted expected protein percentage during periods when Bovaer was not administered. The average difference is 0.6 ± 0.3 percentage points and is significantly different from 0 ($P = 0.03$). The data (histogram) are plotted against a normal distribution (curve).

Table 4. Output from GLMSELECT analysis of the Bovaer effect on protein percentage in milk samples from the yield control. The data represent the differences between the protein percentage achieved during the period when Bovaer was administered and the seasonally adjusted expected protein percentage during periods when Bovaer was not administered.

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Value
Model	4	95.33685	23.83421	5.53
Error	68	292.88813	4.30718	
Corrected Total	72	388.22499		
Model Fit Statistics				
Statistic	Value			
Root MSE	2.07537			
Dependent Mean	0.60676			
R-Square	0.2456			
Adj R-Sq	0.2012			
AIC	186.42119			
AICC	187.69391			
SBC	122.87348			
Parameter Estimates				
Parameter	DF	Estimate	Standard Error	t-value
Intercept	1	5.695831	1.876841	3.03
White Clover	1	-35.176569	11.388417	-3.09
PropTotalCanol	1	11.424019	4.170205	2.74
RaceVariable Jersey	1	-1.448444	0.624751	-2.32
RaceVariable Large	0	0	.	.
Ca_gkgDM	1	-0.676687	0.225320	-3.00

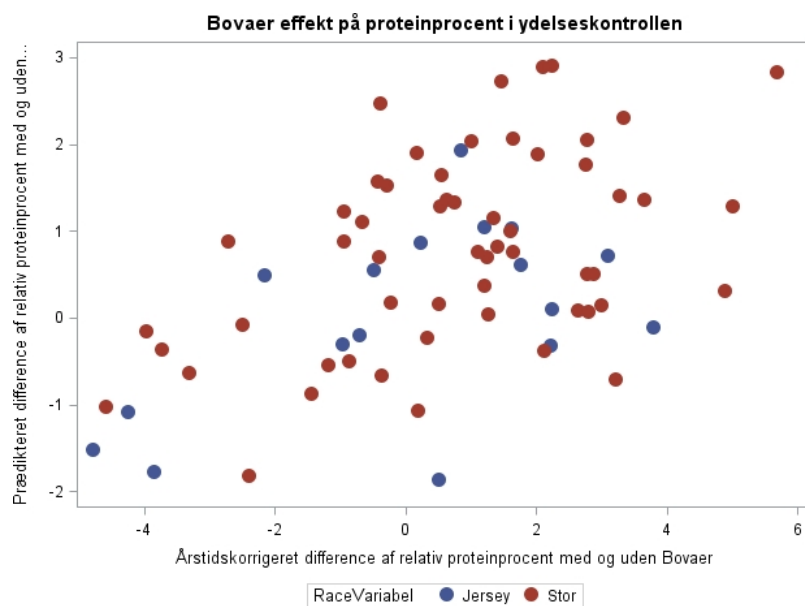


Figure 9. Predicted Bovaer effect on protein percentage from the yield control based on the model presented in Table 4. The model explains 25% of the variation in the dataset.

The addition of Bovaer affected the protein content of the milk, based on analysis of milk samples from the yield checks, with a small increase corresponding to a relative effect of 0.6%. The majority (75%) of the variation in the effect of Bovaer between herds could not be explained, but white clover concentration, calcium concentration, breed and rapeseed in the feed ration explained 25% of the variation.

Response analysis – yield control data – urea

Data on urea concentration from the performance monitoring were modelled as described above for EKM performance, based on Wilmlink (1987). Figure 10 shows the variation throughout the year 2025 based on periods without the allocation of Bovaer in the participating herds. It should be noted that the variation over the year does not appear as a simple seasonal effect, and the variation is likely due to a combination of seasonal effects and recalibrations of the analysis. It should be noted that there is a peak in October for herds that do not feed Bovaer in October.

The relative urea concentration, calculated as the average across all lactations and adjusted for seasonal variation, was strongly influenced by Bovaer and was highly significantly different from 0 ($6.2 \pm 1.2\%$, $P < 0.001$); see Figure 11. This effect is evident from the difference between the relative urea concentration in milk when feeding Bovaer and the data shown in Figure 10.

Application of PROC GLMSELECT and the Schwarz Bayesian information criterion (SBC) to the data set for urea concentration with average deviations (across lactation numbers) within the herd showed a positive effect of white clover on the parameter estimate. The model explained only 12% of the variation in the dataset, and the Bovaer effect was therefore largely unexplained (Figure 12).

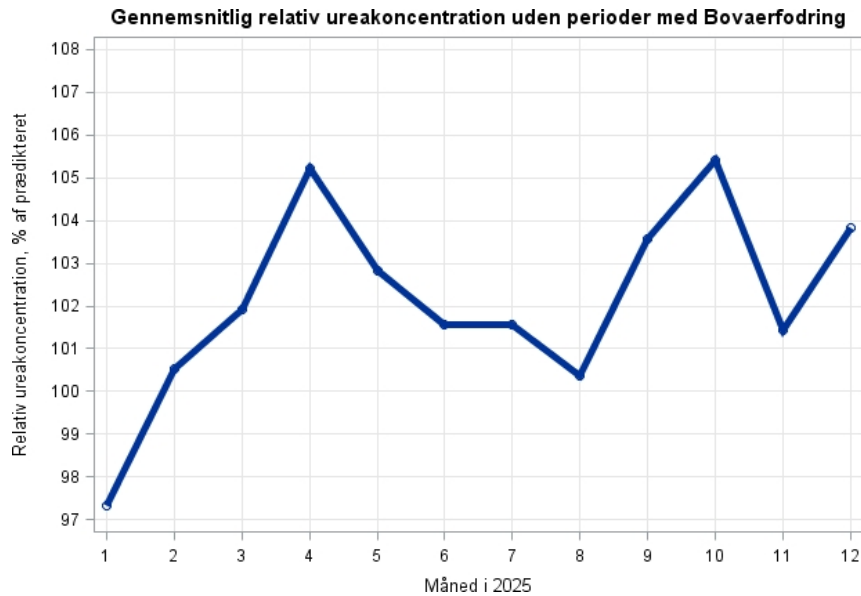


Figure 10. Urea concentration in milk samples from the yield checks relative to predicted urea concentration based on periods without Bovaer administration.

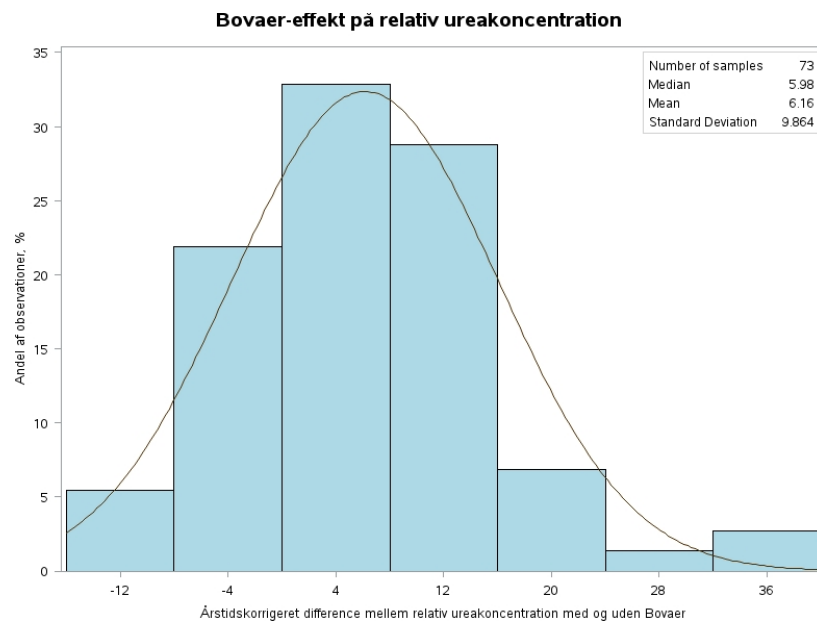


Figure 11. The distribution of differences between the actual urea concentration during periods when Bovaer was administered and the seasonally adjusted expected urea concentration based on all herds during periods when Bovaer was not administered. The average difference was $6.2 \pm 1.2\%$ and significantly different from 0 ($P < 0.001$). The data (histogram) are plotted against a normal distribution (curve).

Table 5. Output from GLMSELECT analysis of the Bovaer effect on urea concentration in milk samples from the yield checks. The data represent differences between values obtained during the period of Bovaer administration and seasonally adjusted expected values during periods without Bovaer administration.

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Value
Model	1	851.61830	851.61830	9.83
Error	71	6153.40774	86.66771	
Corrected Total	72	7005.02604		
Model Fit Statistics				
Statistic	Value			
Root MSE	9.30955			
Dependent Mean	6.16427			
R-Square	0.1216			
Adj R-Sq	0.1092			
AIC	402.70404			
AICC	403.05186			
SBC	332.28496			
Parameter Estimates				
Parameter	DF	Estimate	Standard Error	t-value
Intercept	1	0.593279	2.084634	0.28
White Clover	1	154.199103	49.191269	3.13

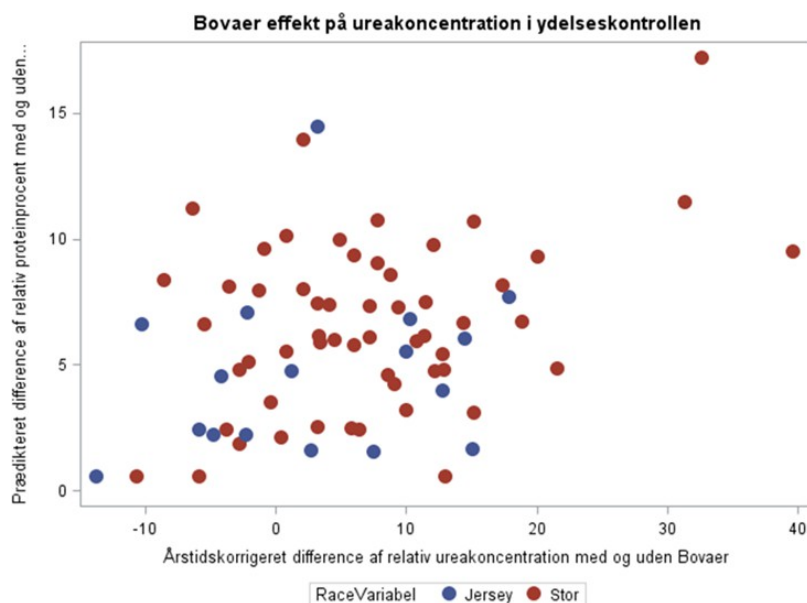


Figure 12. The predicted Bovaer effect on relative urea concentration, plotted against the observed effect, based on a model (Table 5) describing the Bovaer effect on urea in relation to the white clover content of the ration. Only 12% of the variation in the dataset is explained by the model.

The white clover concentration in the feed rations used has been determined with uncertainty, and as the study is retrospective, it has generally not been possible to collect feed samples that represented the feeding practices in the relevant periods within the herds. The reason white clover has nevertheless been included in the study is that it is one of the few significant differences between feed rations in Denmark and those countries where there is also experience of using Bovaer. In Denmark, clover-grass is generally grown for both haymaking and grazing.

White clover contains varying amounts of cyanide bound in glycosides, but so do other forage plants, such as maize (Humphreys, 1988). There is currently no specific hypothesis relating to white clover, but both the fact that white clover in feed for dairy cattle is a factor distinguishing Denmark from countries where fewer problems with Bovaer have reportedly been found than in Denmark, and the fact that a rough estimate of white clover content in the rations is significant in the present study, indicate a need to investigate any effect of clover in more detail. The use of red clover is also, to a large extent, a feature specific to Danish dairy cattle feeding.

Response analysis – yield monitoring data – 3-hydroxybutyrate

Data for 3-hydroxybutyrate concentration from the performance monitoring were modelled as described above for EKM yield based on Wilmink (1987). Figure 13 shows the variation throughout the year 2025 based on periods without the administration of Bovaer in the participating herds. It should be noted that the relative variation in 3-hydroxybutyrate throughout the year is very large compared to fat, protein and urea.

The composition of milk in data from milk yield monitoring has been determined using scanning with an FT-MIR (Fourier-transform mid-infrared spectroscopy) instrument and multivariate prediction models. Since the 1960s, mid-infrared scanning has been used to predict fat and protein in milk samples with high precision, and with the development of FT-MIR it has also become possible to predict urea, albeit with lower precision (Hansen, 1998). The concentration of 3-hydroxybutyrate is likely to be predicted with far less certainty than the prediction of fat and protein. It cannot therefore be expected that a reading for 3-hydroxybutyrate actually indicates a changed concentration of this specific metabolite, but must be regarded as an abstract parameter driven by spectral variation modelled as 3-hydroxybutyrate (Grelet et al., 2016).

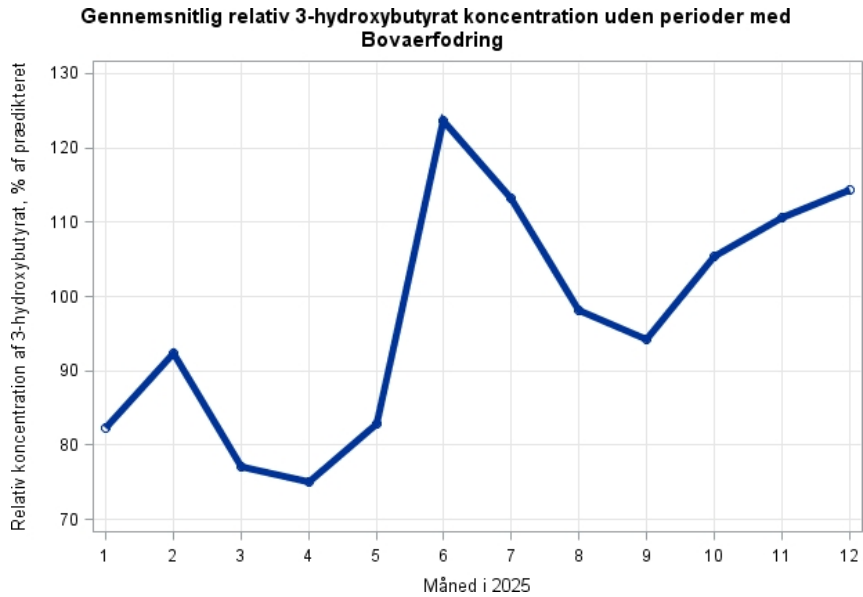


Figure 13. 3-hydroxybutyrate concentration in milk samples from the performance test relative to predicted concentration based on periods without Bovaer administration.

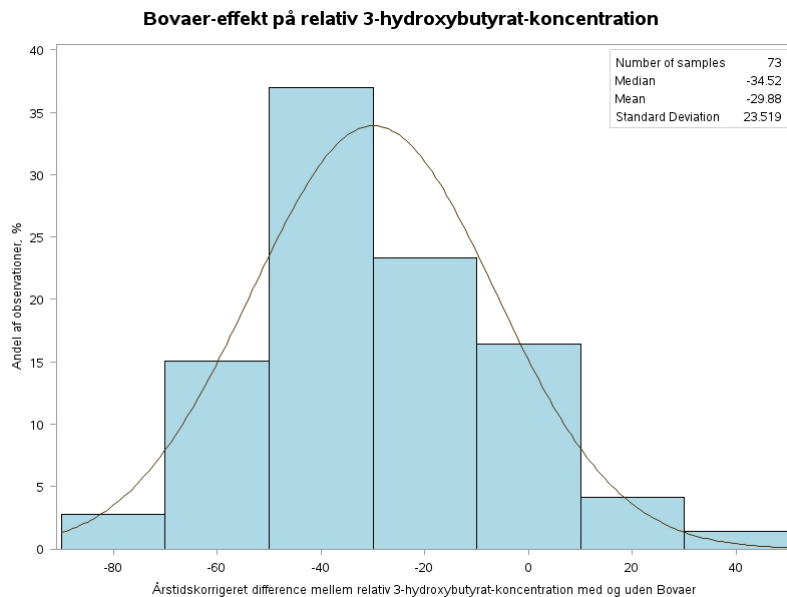


Figure 14. The distribution of differences between the 3-hydroxybutyrate levels achieved during the period when Bovaer was administered and the seasonally adjusted expected concentration based on all herds during periods when Bovaer was not administered. The average difference was $-30 \pm 3\%$ and highly significantly different from 0 ($P < 0.001$). The data (histogram) are plotted against a normal distribution (curve).

The relative 3-hydroxybutyrate concentration, as the average of all lactations adjusted for seasonal variation, was strongly influenced by Bovaer and significantly different from 0 ($-30 \pm 3\%$, $P < 0.001$), see Figure 14.

Application of PROC GLMSELECT and the Schwarz Bayesian information criterion (SBC) to the 3-hydroxybutyrate dataset, with average deviations (across lactation numbers) within the herd

showed the effect of rapeseed meal, CAB value, calcium, cobalt and herd age described by the number of lactation years (Table 6). The model explained 40% of the variation in the dataset (Figure 15).

The uncertainty in the analytical method for 3-hydroxybutyrate means that the effect on 3-hydroxybutyrate must be interpreted with caution, and it cannot be concluded that there is a specific effect on 3-hydroxybutyrate. However, the data indicate, at a minimum, an interaction between ration characteristics and feeding with Bovaer, which has a spectral signature in milk analysed by FT-MIR.

Table 6. Output from GLMSELECT analysis of the Bovaer effect on 3-hydroxybutyrate concentration in milk samples from the performance testing. Data represent differences between values obtained during the period of Bovaer administration and seasonally adjusted expected values during periods without Bovaer administration.

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Value
Model	5	15943	3188.63025	8.94
Error	67	23884	356.47740	
Corrected Total	72	39827		
Model Fit Statistics				
Statistic	Value			
Root MSE	18.88061			
Dependent Mean	-29.87767			
R-Square	0.4003			
Adj R-Sq	0.3556			
AIC	509.70679			
AICC	511.42987			
SBC	448.44955			
Parameter Estimates				
Parameter	DF	Estimate	Standard Error	t-value
Intercept	1	-148.935346	24.918658	-5.98
PropCanolCake	1	-170.862147	56.310853	-3.03
CAB_meqkgDM	1	0.161387	0.046006	3.51
Ca_gkgDM	1	6.481996	2.025370	3.20
Co_mgkgDM	1	32.382813	14.714942	2.20
Milking Year	1	12.133819	4.318977	2.81

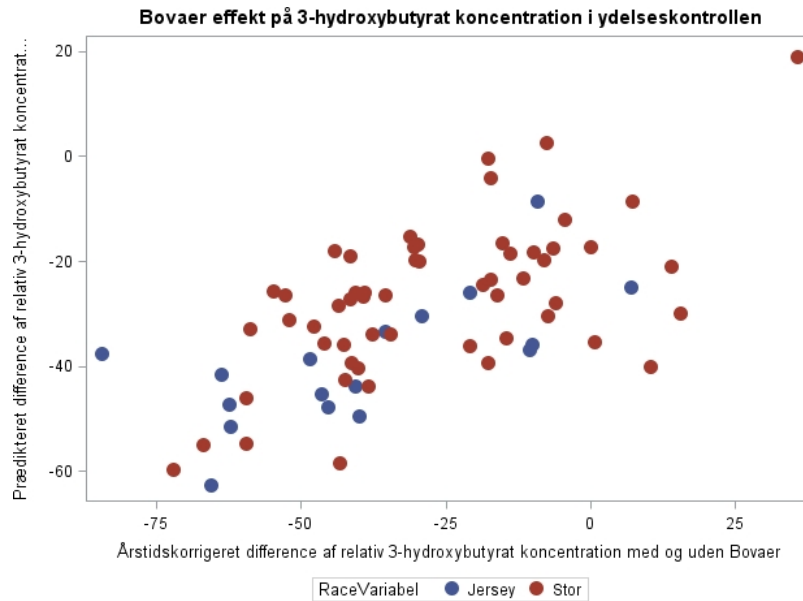


Figure 15. The predicted Bovaer effect on 3-hydroxybutyrate in milk samples, plotted against the observed effect, based on a model (Table 6) describing the Bovaer effect in relation to the ration's content of rapeseed meal, CAB (acid-base balance), calcium and cobalt concentrations, and a variable describing the herd's age. The model explains 40% of the variation in the dataset.

Response analysis – performance testing data – somatic cell count in milk

Cell count data from the performance tests were logarithmically transformed and modelled as described above for EKM performance, based on Wilmlink (1987). Figure 16 shows the variation over the year 2025, based on periods without the allocation of Bovaer in the participating herds. The scale on the y-axis in Figure 16 is the same as that used for fat percentage in Figure 5, which highlights the fact that the seasonal effect on cell count was far less influenced by the season in 2025 compared with fat percentage. It is also noted that cell counts were generally low in 2025 compared with previous years.

Data for relative logarithmically transformed cell counts, calculated as the average across all lactations and adjusted for annual-seasonal variation, were not influenced by Bovaer and did not differ from 0 (-0.5 ± 0.6 %, $P = 0.36$); see Figure 17.

Application of PROC GLMSELECT and the Schwarz Bayesian information criterion (SBC) to the dataset for cell counts with mean deviations (across lactation numbers) within herds revealed a positive effect of starch concentration in the feed ration, as indicated by a positive sign for the parameter estimate (Table 7). The model explained 11% of the variation in the dataset, and the variation in response to Bova tests between herds was therefore largely unexplained.

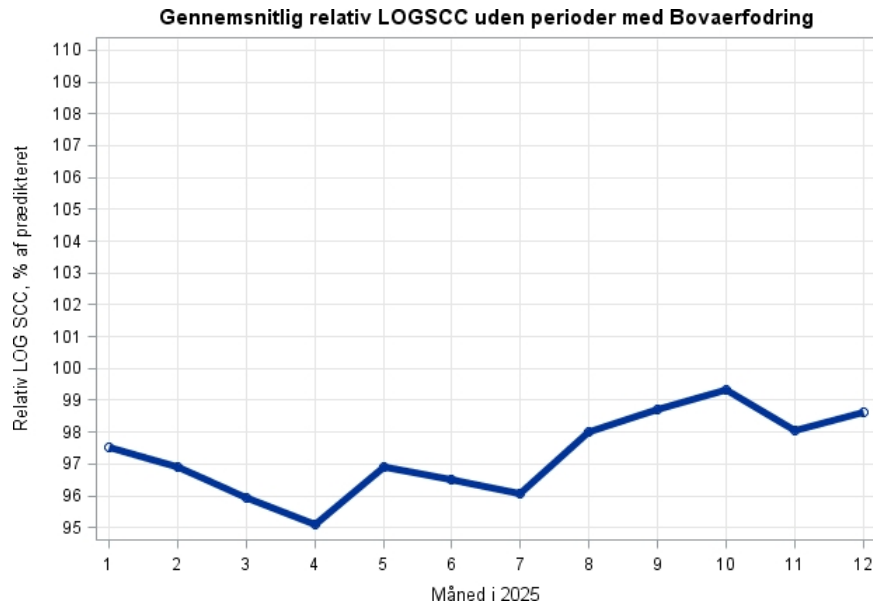


Figure 16. Log-transformed cell counts in milk samples from the performance testing relative to predicted values based on periods without Bovaer administration.

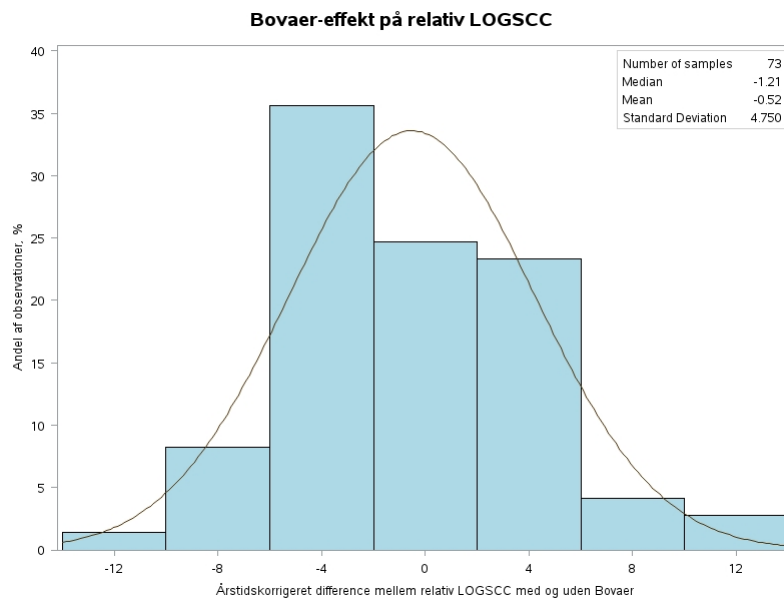


Figure 17. The distribution of differences between the observed logarithm-transformed cell count during the period of Bovaer allocation and the seasonally adjusted expected value based on all herds during periods without Bovaer allocation. The average difference was not significantly different from 0 – $0.5 \pm 0.6\%$ ($P = 0.36$). The data (histogram) are plotted against a normal distribution (curve).

Table 7. Output from GLMSELECT analysis of the Bovaer effect on logarithm-transformed cell counts in milk samples from the performance tests. The data represent differences between values obtained during the period of Bovaer administration and seasonally adjusted expected values during periods without Bovaer administration.

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Value
Model	1	171.89766	171.89766	8.30
Error	71	1463.29463	20.60978	
Corrected Total	72	1635.19229		
Model Fit Statistics				
Statistic	Value			
Root MSE	4.53980			
Dependent Mean	-0.48836			
R-squared	0.1051			
Adj R-Sq	0.0925			
AIC	297.85300			
AICC	298.20083			
SBC	227.43392			
Parameter Estimates				
Parameter	DF	Estimate	Standard Error	t-value
Intercept	1	-12.497856	4.192212	-2.98
ST_gkgDM	1	0.060258	0.020865	2.89

Summary of data from the performance test

Using data from the performance monitoring, the herds' data from 2025 were assessed against models optimised with data from the years 2022, 2023 and 2024. The analyses of Bovaer effects were carried out relative to expected effects based on previous years and seasonal variation in 2025, based on periods when the herds were not fed Bovaer.

Variables in performance recording data that were not significantly affected by Bovaer

Milk production, calculated as EKM yield over a 305-day lactation period, EKM yield in early lactation (10 to 60 days into lactation), milk fat percentage and milk somatic cell count were not significantly affected by the administration of Bovaer.

Variables in performance testing data that were significantly affected by Bovaer

The protein content of the milk was slightly affected by Bovaer, the urea concentration was moderately affected by Bovaer, and the 3-hydroxybutyrate concentration in the milk was strongly affected by Bovaer. The effects of Bovaer observed were derived from the application of multivariate prediction models to FT-MIR spectra of milk, and it is uncertain whether the prediction models are robust to secondary Bovaer effects that may leave a spectral imprint in the milk. Therefore, it cannot be concluded that it is actually protein, urea

and 3-hydroxybutyrate that were affected, nor can it be concluded that there is a causal effect of the descriptive variables on the Bovaer effect shown in Table 8.

Table 8. Overview of descriptive variables from the performance test influenced by feeding with Bovaer, along with an indication of the effect size of the relative effect of Bovaer. Parameter estimates for the descriptive variables are shown with their signs. Note that only parameter estimates for the descriptive variables influenced by Bovaer have been included.

Response	Descriptive variables							
	Proportion of rapeseed meal in the ration	Proportion of total rapeseed in the ration	White clover content	Breed	Calcium concentration	Cobalt concentration	CAB value	Milking year
Relative protein concentration in milk (+0.6%)		+11.4	-35.2	Jersey -1.4	-0.7			
Relative urea concentration in milk (+6.2%)			+154.2					
Relative 3-hydroxybutyrate concentration in milk (-30%)	-170.9				+6.5	+32.4	+0.2	+12.1

As Bovaer did not affect the cows' milk production or the somatic cell count in the milk, the analysis of data from the milk recording schemes suggests that the overall effect of Bovaer was very small. There are qualitative effects of Bovaer, which may be a response to a more general metabolic effect of Bovaer, potentially driven by an influence on ruminal fermentation.

The absence of a general Bovaer effect on the overall production variables such as milk yield and somatic cell count does not mean that more acute effects of Bovaer cannot occur, but these potential acute effects do not appear to have had a significant impact on the data from the yield monitoring.

Response analysis – milk delivered – fat percentage

The composition of milk (fat, protein and urea concentration) delivered to the dairy and the somatic cell count of this milk were collected at delivery level, meaning that for the vast majority of herds there is a data point for every other day.

An average was calculated for each week, and the data was scaled by dividing each weekly average by the herd's annual average, which was then multiplied by 100 to obtain a scale comparable to data based on the performance test. However, there is a significant difference in the calculation, as the yield monitoring data was compared with a herd model based on data from previous years. The analysis of milk delivery data is based solely on data from 2025. The analysis of milk deliveries was carried out for 71 of the 73 herds, as two herds were fed Bovaer throughout the year, and the scaling of data for these two herds will therefore offset any potential effect of Bovaer.

Figure 18 shows the average of the scaled fat percentage for 71 herds during periods throughout 2025 when Bovaer was not fed. The scaling makes it possible to compare data from Jersey and large breeds, as the correlation between seasonal variation and breed was found to be 0.98 (calculated using PROC CORR in SAS, data not shown).

A similar seasonal variation is observed between the fat percentage variation shown in Figure 5 (for the yield control) and Figure 18, although with higher temporal resolution in Figure 18.

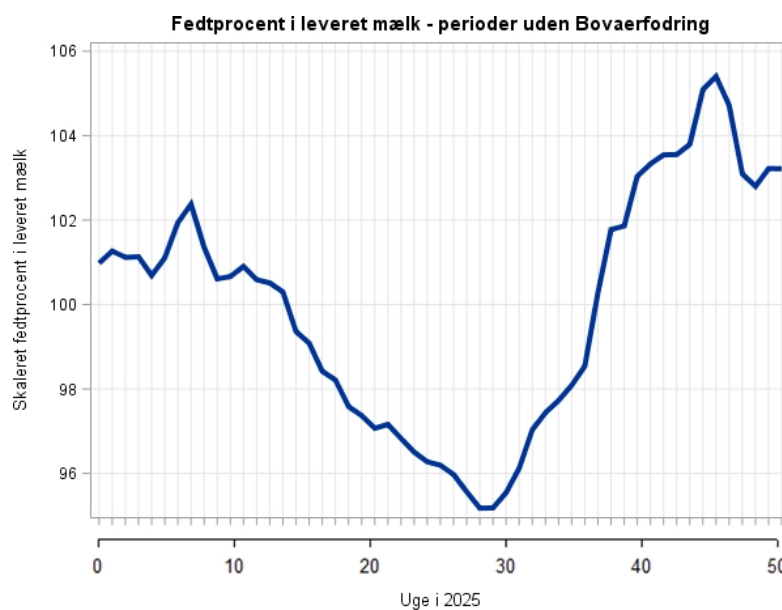


Figure 18. Scaled fat percentage in delivered milk as an average of all herds (both Jersey and large breeds), including periods when Bovaer was not fed. For each herd, a weekly average and an annual average were calculated. Data were scaled by dividing the weekly averages within the herd by the herd's annual average, which was then multiplied by 100. The data are based on 71 herds.

For each herd, a difference was calculated between the scaled fat percentages in weeks when Bovaer was fed and the average scaled fat percentage shown in Figure 18. For each herd, the effect of Bovaer during the period when Bovaer was administered was then calculated as the average of these differences. The distribution of the differences is shown in Figure 19. There was a higher fat percentage in delivered milk during periods of Bovaer administration compared with periods without Bovaer administration, and the difference was significantly different from 0 ($0.7 \pm 0.2\%$, $P < 0.01$).

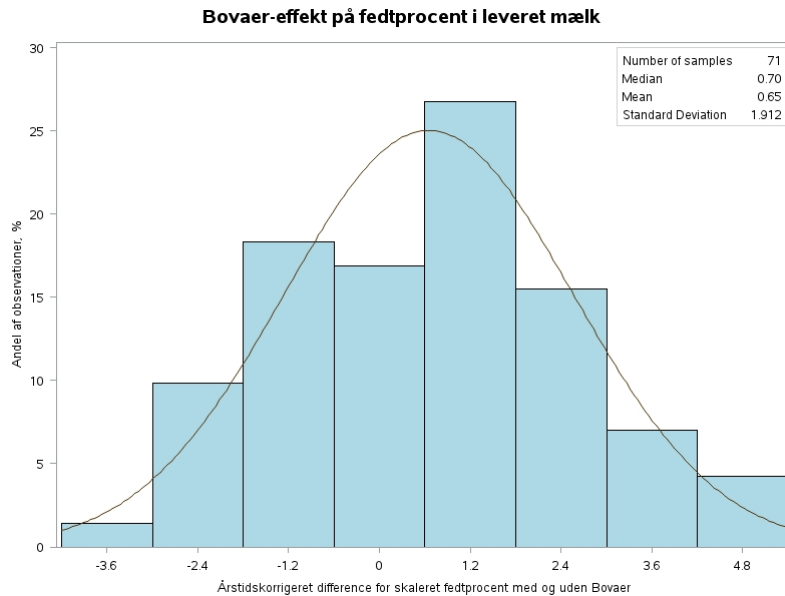


Figure 19. Differences between the scaled fat percentage in delivered milk during the period of Bovaer feeding and the average of all herds that did not feed Bovaer during the period. There are 71 herds behind this average, because two herds were fed Bovaer throughout 2025. The effect of Bovaer is significantly different from 0 ($0.7 \pm 0.2\%$, $P < 0.01$). The data (the histogram) are plotted against a normal distribution (the curve).

The differences obtained were analysed using stepwise regression analysis with the PROC GLMSELECT procedure in SAS. To minimise the risk of over-parameterisation, SBC was used as a criterion for model selection in addition to the requirement for the significance level of the included parameters.

The analysis (Table 9) resulted in a model with breed and sugar and sodium concentrations in the feed ration as explanatory variables, but only 19% of the variation in the dataset was explained by the model (Figure 20).

When calculating fat percentage in data from the yield checks, no general effect of Bovaer was detected, but a large number of descriptive variables had an effect on the observed differences in fat percentage. For delivered milk, an effect of Bovaer was detected, but conversely, only a few of the descriptive variables had an effect on the differences, and they provided a very limited explanation of the effect of Bovaer with the optimised model. It is possible that the higher temporal resolution in the milk delivery data meant that the analysis was more sensitive in explaining the effect of Bovaer, but by far the largest part of the variation between herds in the response to Bovaer remains unexplained.

Table 9. Output from GLMSELECT analysis of the Bovaer effect on scaled fat percentage from delivered milk.

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Value
Model	3	48.52066	16.17355	5.22
Error	67	207.42544	3.09590	
Corrected Total	70	255.94610		
Model Fit Statistics				
Statistic	Value			
Root MSE	1.75952			
Dependent Mean	0.65054			
R-Square	0.1896			
Adj R-Sq	0.1533			
AIC	157.11854			
AICC	158.04161			
SBC	93.16926			
Parameter Estimates				
Parameter	DF	Estimate	Standard Error	t-value
Intercept	1	1.947412	1.321132	1.47
RaceVariable Jersey	1	-1.221322	0.508245	-2.40
RaceVariable Large	0	0	.	.
SUG_gkgDM	1	-0.054593	0.021897	-2.49
Na_gkgDM	1	0.434841	0.176257	2.47

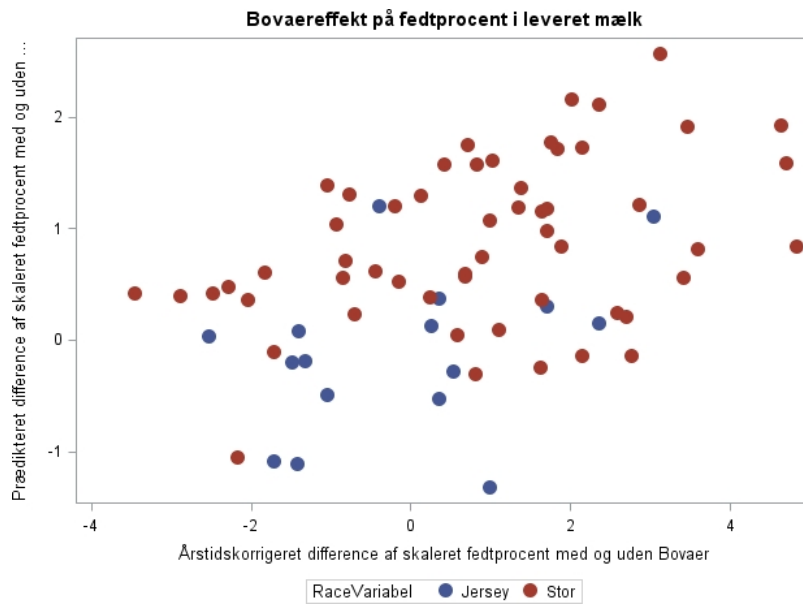


Figure 20. The predicted Bovaer effect on scaled fat percentage in delivered milk, plotted against the observed effect, based on a model (Table 9) describing the Bovaer effect by breed as well as sugar and sodium concentration in the feed ration. The model explains 19% of the variation in the dataset.

Response analysis – milk yield – protein percentage

Data for protein percentage in delivered milk were treated as described for fat percentage above. The seasonal variation in scaled protein percentage during periods without Bovaer allocation is shown in Figure 21. It should be noted that, for protein percentage, there is a consistent profile between data from the yield recording (Figure 7) and delivered milk (Figure 21).

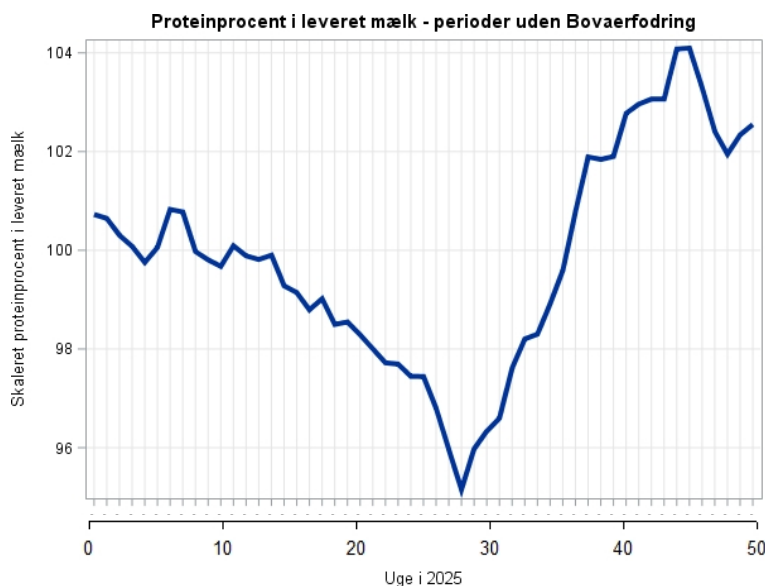


Figure 21. Scaled protein percentage in delivered milk as the average of all herds (both Jersey and large breeds), including periods when Bovaer was not fed. For each herd, a weekly average and an annual average were calculated. Data were scaled by dividing the weekly averages within the herd by the herd's annual average, which was then multiplied by 100. The data is based on 71 herds.

Figure 22 shows the distribution of differences between the scaled protein percentage in delivered milk and the expected value shown in Figure 21. A strong significant effect of Bovaer ($P < 0.001$) was found, with higher protein concentrations than expected during periods of Bovaer administration (the scaled difference was $1.0 \pm 0.1\%$ higher with Bovaer administration). This is consistent with the fact that an effect of Bovaer on protein percentage was also detected in data from the milk recording scheme.

Modelling the response to Bovaer (in terms of protein percentage in delivered milk) across herds resulted in a model with a single explanatory variable, the Bovaer dose variable (Table 10), which is a weighted dose indicator of the herds' Bovaer allocation. A higher Bovaer dose resulted in a higher protein percentage. The model explains only a small proportion of the variation, 8%, between herds and is therefore an inadequate description of the effect of Bovaer (Figure 23). It should be noted that the model optimised to describe the protein effect in performance testing data does not include the variable 'Bovaer dose', but instead includes descriptive variables that were not significant in the model for protein percentage in delivered milk. Both models describe only a small part of the variation in the dataset, and it is therefore possible that other significant factors have not been identified, which are not included in the descriptive variables in the study, or that the data contain a significant element of analytical or random variation (noise) that cannot be described. However, it is important to note that a Bovaer effect on the protein percentage in milk analysed by FT-MIR was identified in both the yield monitoring data and the milk delivery data.

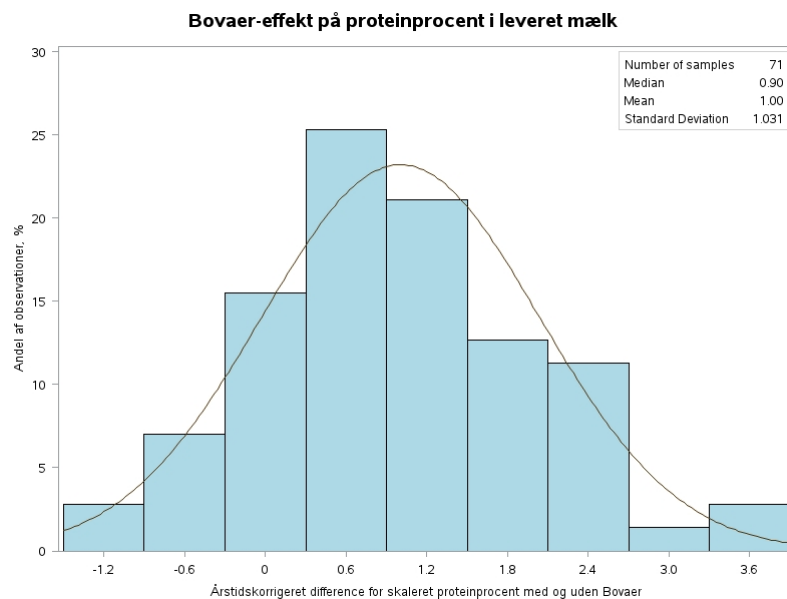


Figure 22. Differences between the scaled protein percentage in delivered milk during the period of Bovaer feeding and the average of all herds that did not feed Bovaer during the period. There are 71 herds in the dataset because two herds fed Bovaer throughout 2025. A strong significant effect of Bovaer ($P < 0.001$) was found, with higher protein concentrations than expected during periods of Bovaer administration (the scaled difference was $1.0 \pm 0.1\%$). The data (the histogram) are plotted against a normal distribution (the curve).

Table 10. Results from the GLMSELECT analysis of the Bovaer effect on the scaled protein percentage of delivered milk.

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Value
Model	1	6.26176	6.26176	6.34
Error	69	68.14922	0.98767	
Corrected Total	70	74.41097		
Model Fit Statistics				
Statistic	Value			
Root MSE	0.99382			
Dependent Mean	1.00483			
R-Square	0.0842			
Adj R-Sq	0.0709			
AIC	74.09040			
AICC	74.44861			
SBC	5.61576			
Parameter Estimates				
Parameter	DF	Estimate	Standard Error	t-value
Intercept	1	-0.407949	0.573350	-0.71
BovaerDosisScore	1	0.016310	0.006478	2.52

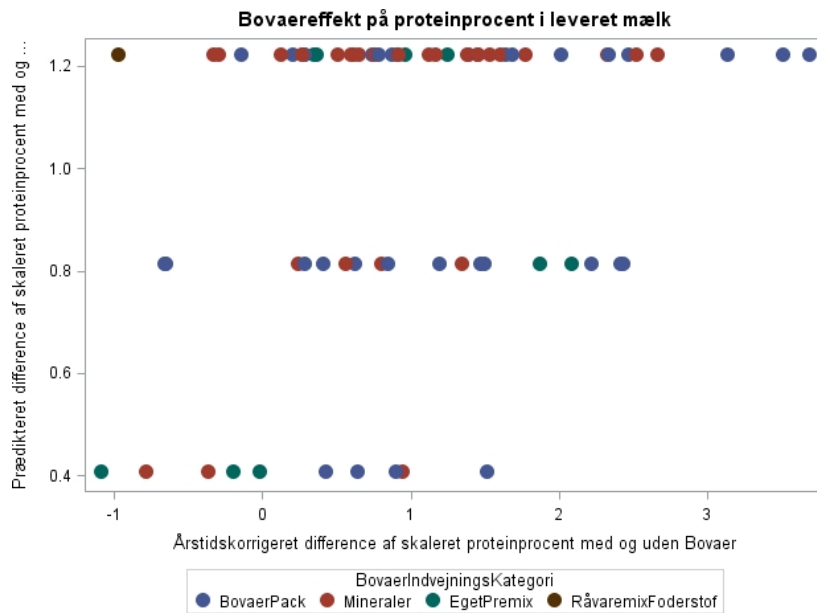


Figure 23. The predicted Bovaer effect on the scaled protein percentage in delivered milk, plotted against the observed effect, based on a model (Table 10) that describes the Bovaer effect using a weighted allocation of Bovaer (a numerical variable with values of 50, 75 or 100). The model explains only 8% of the variation in the dataset.

Response analysis – delivered milk – urea concentration

Data for urea concentration in delivered milk were not scaled as fat and protein percentages, but used without pre-processing. There is no marked seasonal variation in urea concentration as found for fat and protein percentages (Figure 24), which is consistent with the urea concentration in milk samples from the yield control (Figure 10).

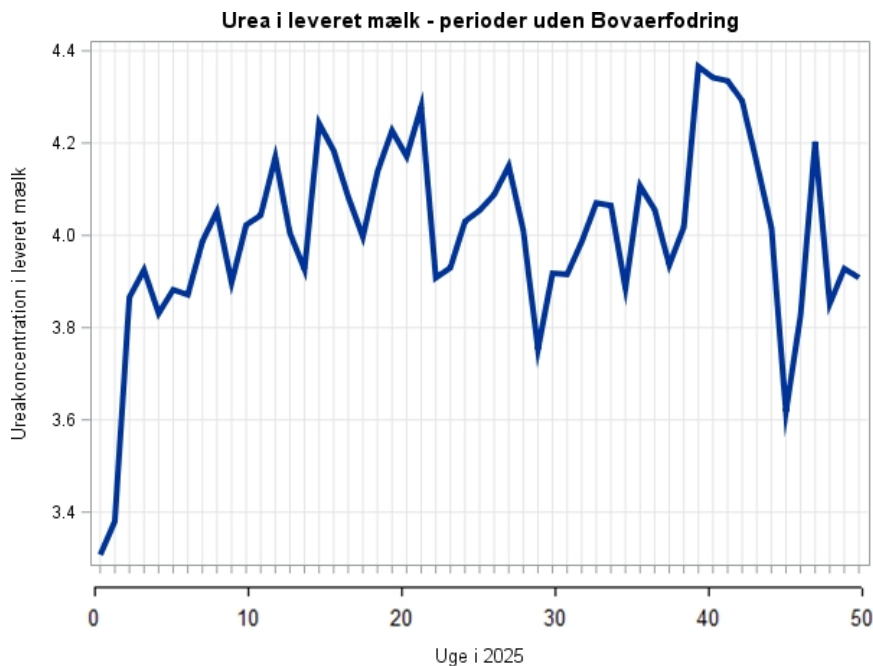


Figure 24. Urea concentration (mM) in delivered milk as the average of all herds (both Jersey and large breeds), including periods when Bovaer was not fed.

Differences in urea in delivered milk were influenced by feeding with Bovaer, with an average difference of 0.18 ± 0.05 mM ($P < 0.001$; Figure 25).

The results of a stepwise regression analysis using PROC GLMSELECT in SAS on data for urea differences are shown in Table 11. The optimised model explained 35% of the variation in the dataset using breed, forage proportion and crude protein concentration (CP) in the feed ration. The predicted versus observed values are shown in Figure 26. A significant effect of Bovaer on urea was also observed in the analysis of performance testing data. For urea, the modelling of the response also resulted in models with different explanatory variables, and as the models simultaneously explain only a small proportion of the variation in the datasets, the significance of the explanatory variables must be interpreted with caution. It is quite possible that the descriptive variables emerging from the optimisation of the models actually point to other underlying conditions in the herds that are not described.

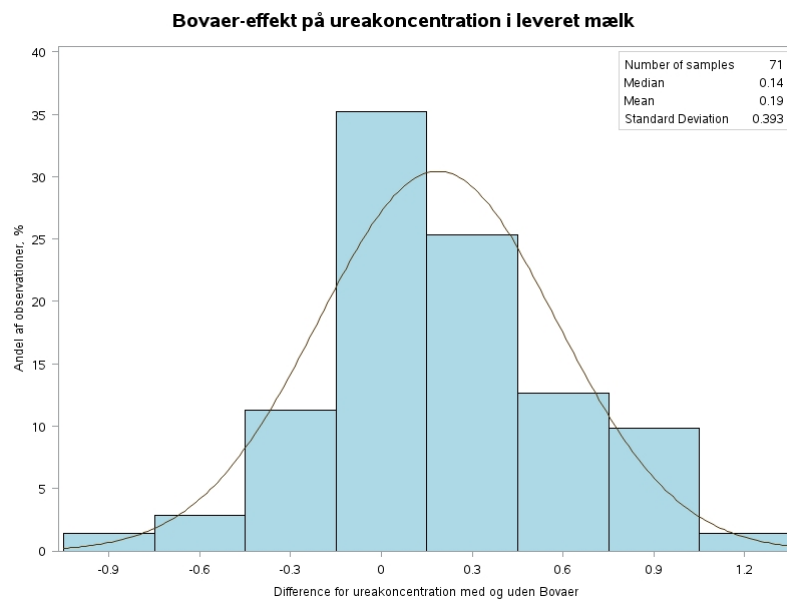


Figure 25. Differences between urea concentrations in milk during the period when Bovaer was fed and the average for all herds that did not feed Bovaer during that period. There are 71 herds in the dataset because two herds fed Bovaer throughout 2025. The overall effect of Bovaer was 0.18 ± 0.05 mM ($P < 0.001$). The data (histogram) are plotted against a normal distribution (curve).

Table 11. Output from GLMSELECT analysis of the effect of Bovaer on urea concentration in delivered milk.

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Value
Model	3	3.75830	1.25277	11.87
Error	67	7.07046	0.10553	
Corrected Total	70	10.82875		
Model Fit Statistics				
Statistic	Value			
Root MSE	0.32485			
Dependent Mean	0.18585			
R-Square	0.3471			
Adj R-Sq	0.3178			
AIC	-82.77960			
AICC	-81.85653			
SBC	-146.72888			
Parameter Estimates				
Parameter	DF	Estimate	Standard Error	t-value
Intercept	1	-7.038599	1.525251	-4.61
RaceVariable Jersey	1	-0.343798	0.097143	-3.54
RaceVariable Large	0	0	.	.
Forage_pctDM	1	0.020186	0.007350	2.75
CP_gkgDM	1	0.036993	0.007786	4.75

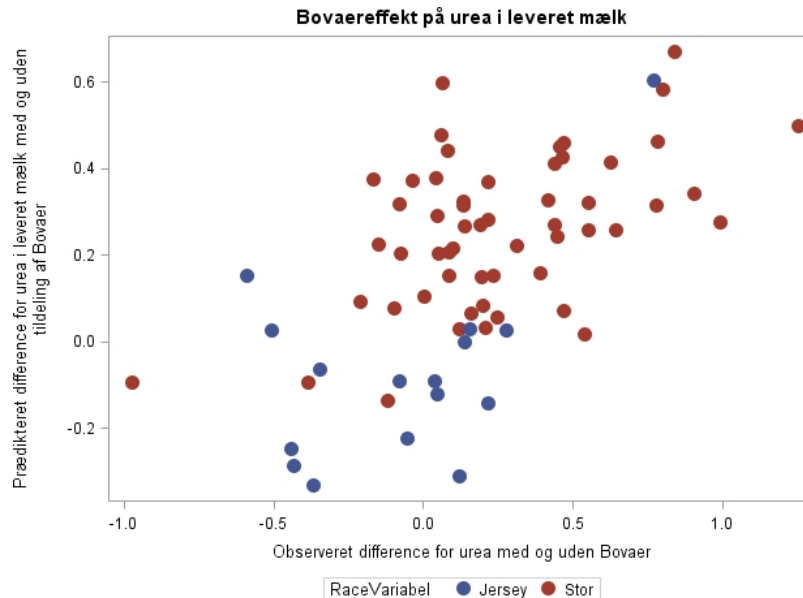


Figure 26. The predicted Bovaer effect on urea in delivered milk, plotted against the observed effect, based on a model (Table 11) describing the Bovaer effect by breed as well as the proportion of roughage and the crude protein concentration in the feed ration. The model explains 35% of the variation in the dataset.

Response analysis – delivered milk – somatic cell count

Data on cell counts in delivered milk were logarithmically transformed and scaled as described for fat content above. The seasonal variation in scaled cell count over periods throughout 2025 without Bovaer allocation is shown in Figure 27. It should be noted that the profile for delivered milk differs from that of the yield checks (Figure 16), but for both profiles the relative variation is within a few per cent.

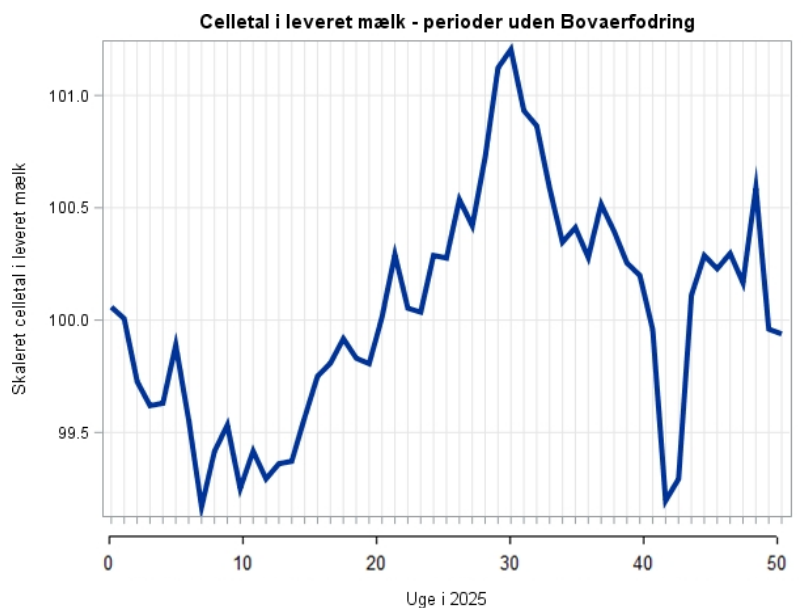


Figure 27. Scaled cell count (log-transformed) in delivered milk as an average of all herds (both Jersey and large breeds), including periods when Bovaer was not fed. For each herd, a weekly average and an annual average were calculated. Data were scaled by dividing the weekly averages within the herd by the herd's annual average and then multiplying by 100. Data are based on 71 herds.

Feeding Bovaer did not affect the somatic cell count in milk; the average difference with and without Bovaer was -0.12 ± 0.10 ($P = 0.20$; Figure 28).

The results of a stepwise regression analysis using PROC GLMSELECT in SAS on data for scaled cell counts are shown in Table 12. The optimised model explained 18% of the variation in the dataset based solely on the description of the rear edges of the feed troughs (Figure 29).

Cell counts in delivered milk were generally not influenced by Bovaer, and the result is consistent with the analysis of cell counts from the milk recording scheme. Modelling the variation between herds resulted in models with poor description of the variation in the dataset and different explanatory variables (starch in milk recording data and feed trough edges for delivered milk, respectively).

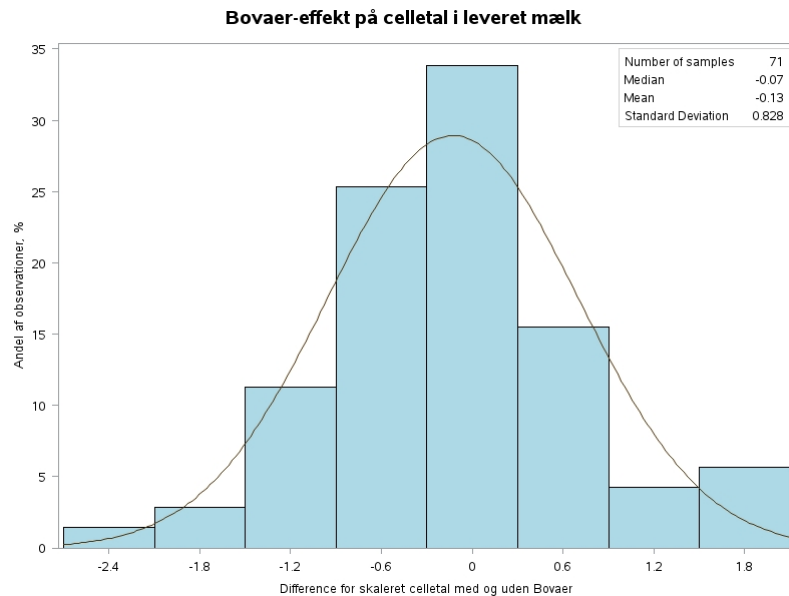


Figure 28. Differences in somatic cell counts in delivered milk during the period of Bovaer feeding and the average of all herds that did not feed Bovaer during the period. There are 71 herds in the dataset because two herds were fed Bovaer throughout 2025. Feeding with Bovaer had no overall effect on the somatic cell count in delivered milk (-0.12 ± 0.10 , $P = 0.20$). The data (the histogram) are plotted against a normal distribution (the curve).

Table 12. Output from GLMSELECT analysis of the Bovaer effect on scaled cell count in delivered milk.

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F-value
Model	2	8.82929	4.41464	7.68
Error	68	39.11257	0.57518	
Corrected Total	70	47.94186		
Model Fit Statistics				
Statistic	Value			
Root MSE	0.75841			
Dependent Mean	-0.12747			
R-Square	0.1842			
Adj R-Sq	0.1602			
AIC	36.66724			
AICC	37.27330			
SBC	-29.54472			
Parameter Estimates				
Parameter	DF	Estimate	Standard Error	t-value
Intercept	1	-0.324518	0.169585	-1.91
Rear edge of crib High	1	-0.051794	0.218934	-0.24
Low rear edge	1	0.740190	0.236958	3.12
Crib back edge Medium	0	0	.	.

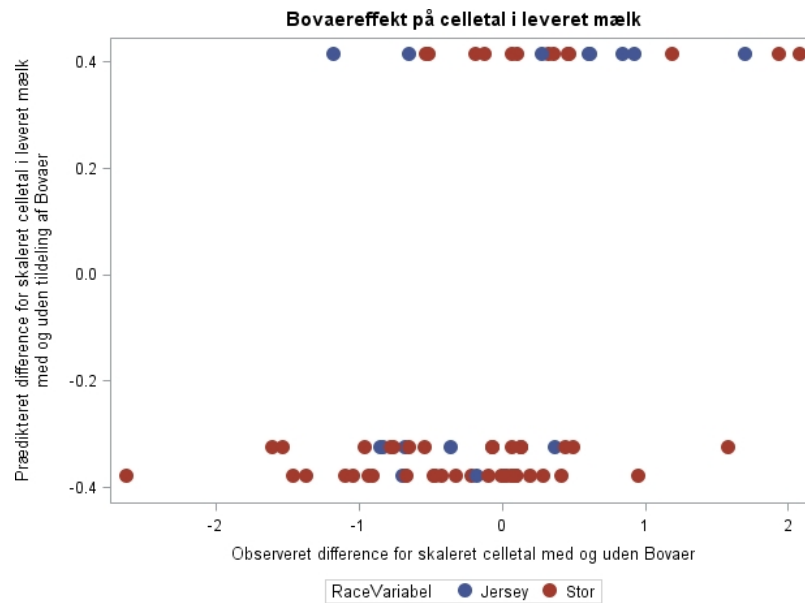


Figure 29. The predicted Bovaer effect on scaled cell count in delivered milk, plotted against the observed effect, based on a model describing the Bovaer effect of feed trough edges. The model explains 18% of the variation in the dataset.

Summary of data for delivered milk

Using data from milk deliveries in 2025, the effect of Bovaer was analysed as differences between periods with Bovaer supplementation and the average composition of milk across herds that did not feed Bovaer during the same period. The analysis includes 71 herds instead of 73, because two herds fed Bovaer throughout the year.

Variables in the data for milk delivered that were not significantly affected by Bovaer

The somatic cell count in delivered milk was not affected by Bovaer feeding. This result is consistent with the analysis of milk samples in the yield monitoring scheme, where no effect of Bovaer was found either.

Variables in delivered milk data that were significantly affected by Bovaer

Fat percentage, protein percentage and urea concentration in delivered milk were affected by feeding with Bovaer. Modelling of the Bovaer response, using the explanatory variables shown in Table 1, explained relatively little of the variation between herds. Models with an intercept and the parameter estimates shown in Table 13 described 19%, 8% and 35% of the variation between herds for the fat, protein and urea responses, respectively.

Table 13. Overview of descriptive variables from milk yield influenced by feeding with Bovaer, indicating the effect size of the relative effect of Bovaer. Parameter estimates for the descriptive variables are shown with signs. Note that only parameter estimates for the descriptive variables influenced by Bovaer are included.

	Descriptive variables					
Response	Breed variable	Bovaer dose score	Roughage proportion	Crude protein in feed ration	Sugar in feed ration	Sodium in the feed ration
Fat content in delivered milk (+0.7%)	Jersey -1.2				-0.05	+0.43
Protein content in delivered milk (+1.0%)		+0.016				
Urea concentration in delivered milk (0.18 mM)	Jersey -0.3		+0.02	+0.04		

Analyses of milk deliveries confirmed the observed effects of Bovaer from yield monitoring data for protein and urea, and in addition, an effect of Bovaer on fat concentration was detected (which was not observed in the yield monitoring data). Somatic cell count was not affected by Bovaer, and as somatic cell count is primarily related to udder infections (Harmon, 1994), it would not immediately be expected, even if Bovaer has a significant effect on the cow's metabolism, that this would manifest itself in altered somatic cell counts. It should be noted that there is also a significant methodological difference between somatic cell count and the other measured parameters, as somatic cell count is determined by flow cytometry, whilst the other parameters are predicted on the basis of an FT-MIR spectrum.

Response analysis – digestive and metabolic disorders

Cows treated for the following disease codes: digestive disorder, acidosis (rumen acidosis), mastitis/toxicity, enteritis, digestive/metabolic disorder (other), other disorders and diarrhoea were recorded by treatment date. Prevalence was calculated on a monthly basis. No indications of a seasonal effect on the treatments were found in the dataset.

Figure 30 shows the differences between periods of Bovaer feeding in individual herds in 2025 and the average for all herds during the same period without Bovaer feeding. There are a few herds with a high prevalence of digestive and metabolic disorders during the period of Bovaer feeding, but there is no overall effect of Bovaer in the dataset ($P = 0.59$, Signed Rank test PROC UNIVARIATE SAS).

With very few observations of herds with an elevated prevalence, it is not possible to analyse the data using a wide range of descriptive variables, as the analysis would simply identify variables that may happen to differ for these few herds with high prevalence.

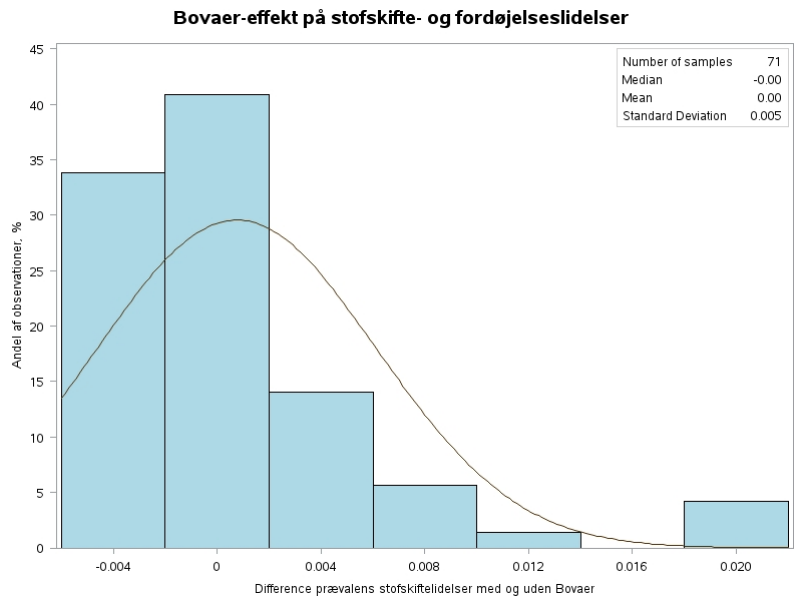


Figure 30. Differences in the prevalence of digestive and metabolic disorders during the period of feeding with Bovaer and the average of all herds that did not feed with Bovaer during the period. There are 71 herds in the dataset because two herds fed with Bovaer throughout 2025. The data (histogram) were compared with a normal distribution (curve), and the test for normal distribution was rejected, $P < 0.01$. Feeding with Bovaer had no overall effect on the prevalence of digestive and metabolic disorders (Signed Rank test $P = 0.59$, PROC UNIVARIATE in SAS).

Response analysis – mastitis

Cows treated with the disease codes mastitis treated with NSAIDs, mastitis, mastitis (acute) and mastitis with paralysis were recorded by treatment date. Prevalence was calculated on a monthly basis. No indications of a seasonal effect on treatments were found in the dataset.

Figure 31 shows the differences between the period of Bovaer feeding in 2025 in individual herds and the average for all herds during the same period without Bovaer feeding. There are a few herds with a high prevalence of mastitis during the period of Bovaer feeding, but there is no overall effect of Bovaer in the dataset ($P = 0.83$, Signed Rank test PROC UNIVARIATE SAS).

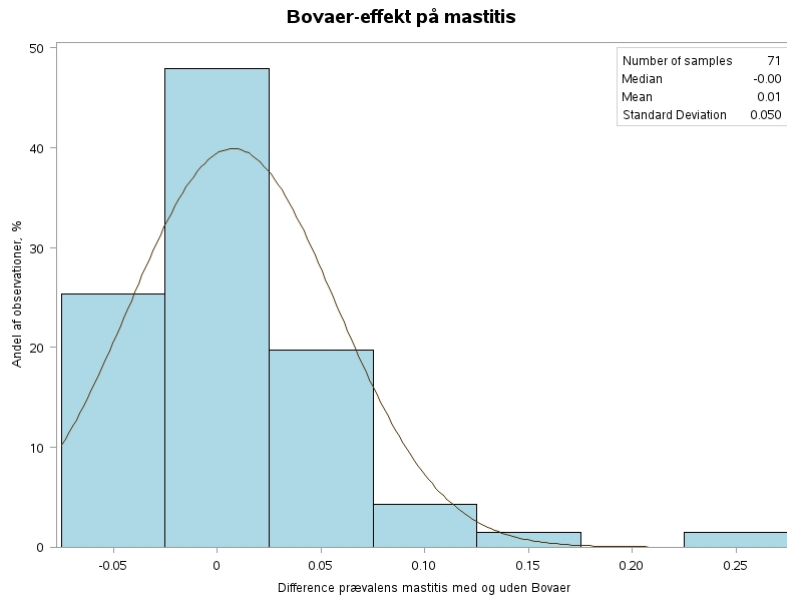


Figure 31. Differences in the prevalence of mastitis during the period of Bovaer feeding and the average for all herds that did not feed Bovaer during that period. There are 71 herds in the dataset because two herds fed Bovaer throughout 2025. The data (histogram) are compared with a normal distribution (curve), and the test for normal distribution was rejected, $P < 0.01$. Feeding with Bovaer had no overall effect on the prevalence of mastitis (Signed Rank test $P = 0.83$, PROC UNIVARIATE in SAS).

Response analysis – mortality

Data on the number of cows that died naturally and were culled in the participating herds were extracted via a report from the DMS cattle management programme. Mortality was calculated on a monthly basis but expressed as the percentage of deaths per year.

No effect of feeding Bovaer was detected on mortality, calculated as the difference between mortality during periods of Bovaer feeding and the average mortality for herds that did not feed Bovaer during the same period (difference $0.17 \pm 0.35\%$, $P = 0.63$; Figure 32, Figure 33).

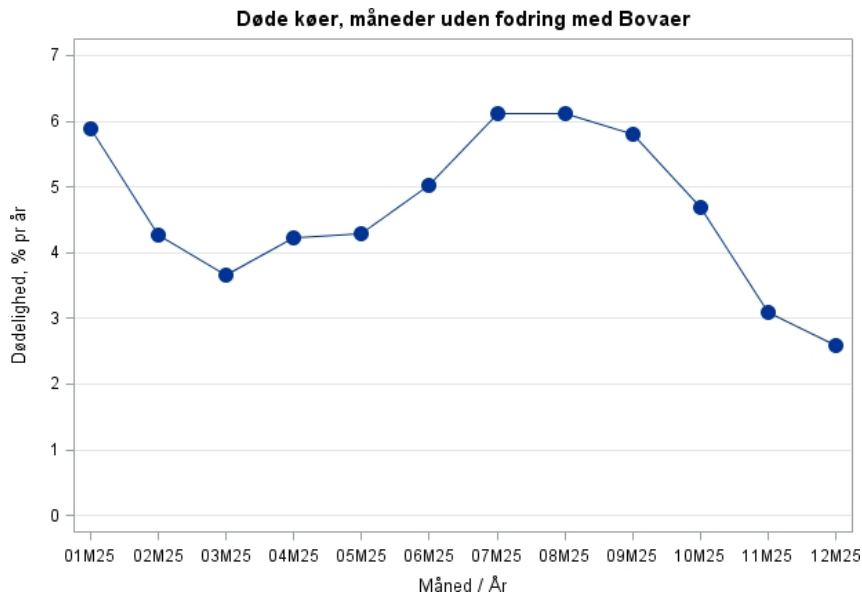


Figure 32. Mortality (% of natural deaths and culled animals per year) as an average of all herds, including periods when Bovaer was not used. The figure shows that, in this dataset, mortality is numerically higher in late summer.

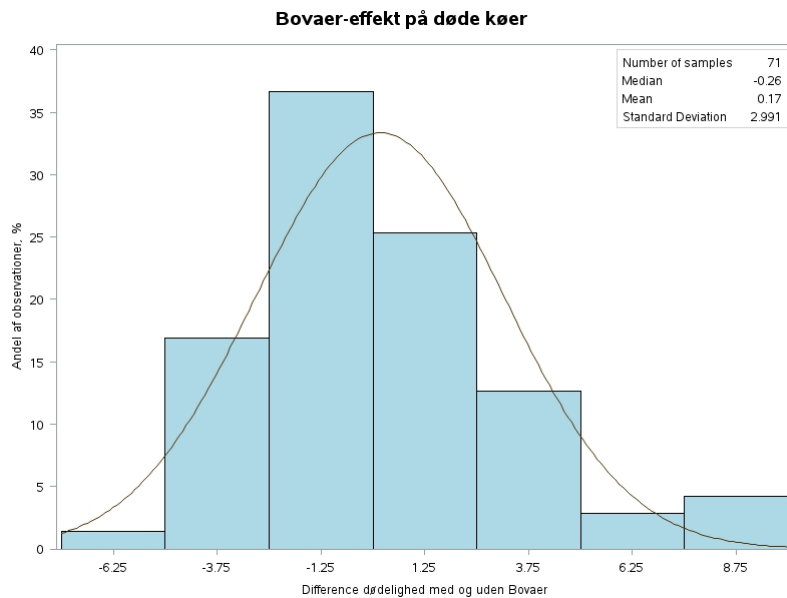


Figure 33. Differences in mortality during the period of Bovaer feeding and the average for all herds that did not feed Bovaer during the period. There are 71 herds in the dataset because two herds were fed Bovaer throughout 2025. The data (the histogram) is compared with a normal distribution (the curve). Feeding with Bovaer had no overall effect on mortality ($P = 0.63$).

Summary of disease treatments and mortality

No overall effect of feeding Bovaer was found on digestive and metabolic disorders, mastitis or mortality in the participating herds.

One of the challenges of the analysis is that many of the cows that have been separated due to reduced rumination, reduced milk yield or health alerts have probably not been treated with prescription medication. These cows have been housed for short periods in a sick pen and may have received supportive treatment such as fluids, hay, activated charcoal or products to stimulate rumen function. Cows that have not been treated with veterinary medicines are not clearly identified in the database, and the extent of such treatments is not available across the herds studied. This study is therefore likely to underestimate the extent of challenges in the category of digestive and metabolic disorders, but it is difficult to assess the degree of uncertainty.

One of the factors supporting the findings of this study, based on recorded treatments and mortality rates, is that the mortality rate for cows nationwide was lower in October and November 2025 than in the two preceding years, despite the fact that a very large proportion of the country's conventional herds were fed Bovaer during precisely these autumn months of 2025 (data not shown).

3. Overall discussion

This study is a retrospective analysis of the potential effects of Bovaer allocation in a sample of 73 Danish dairy herds. Interviews with herd owners and farm managers provided a picture of the problems experienced as a result of Bovaer administration, consistent with the study by SEGES Innovation P/S and press coverage of Bovaer. Despite this apparent consensus, the analytically detectable effects of Bovaer in production data were generally weak.

Milk production and milk composition

Overall, there was no evidence of any negative effects of Bovaer feeding on the cows' milk production, measured as ECM yield, regardless of whether production was assessed on the basis of entire lactations or solely as a measure of production in early lactation. The fact that there is no general effect on ECM yield does not mean that there are no herds that have experienced a drop in production at the same time as introducing Bovaer. Nor does it mean that Bovaer may not have had an effect in these herds. Overall, there are just as many herds that have experienced an increase in milk production coinciding with the introduction of Bovaer as there are herds that have experienced a decline in production. Furthermore, there is a significant seasonal variation in milk production, which may have coincided with the introduction of Bovaer in several of these herds. At the same time, it is noteworthy that the documented negative effects on milk yield in Danish university trials (Lund et al., 2026) do not appear to be reflected in the present study.

It is clear that there is a significant seasonal effect on the composition of milk, and particularly in September and October there is an almost explosive increase in the concentration of milk constituents (see Figures 18 and 21). The increase in milk constituents is accompanied by a reduction in milk volume; in fact, the volume (= milk yield) falls slightly more than the increase in constituents (see Figure 1). One of the challenges in interpreting this data in real time is that milk yield is measured daily if milking robots or milk meters are in use in the milking parlour, and the weighed milk yield is available as soon as the tanker has collected the milk. Consequently, the decline in milk volume is highly visible, whilst the conversion of kg of milk to EKM occurs with a delay, as the milk's composition in most cases awaits laboratory analysis; or, if analysed locally, this is often carried out using slightly less robust analytical methods than those employed at the milk laboratory. It cannot therefore be ruled out that some of the concern regarding the possible negative effects of Bovaer is due to the strong seasonal effect on milk production, which is particularly evident in the autumn. The combination of the seasonal effect and the introduction of Bovaer in a large number of herds, precisely at that time of year when milk volume falls relatively drastically, and a delayed conversion to EKM yield based on nutrient content, may have contributed to self-reinforcing negative publicity and fears regarding Bovaer, which are difficult to substantiate in production and disease data, including cell counts calculated taking the seasonal effect into account.

Although production levels, measured in EKM, were not affected by feeding Bovaer, a number of effects of Bovaer on milk composition were observed, both from analyses carried out in connection with the yield checks and from analyses of tank milk. In the yield checks, the addition of Bovaer to the feed led to an increase in the predicted concentration of protein and urea. In delivered milk, the fat, protein and urea content all increased with the addition of Bovaer. The analyses from the yield checks show a sharp decrease in the predicted concentration of 3-hydroxybutyrate when Bovaer was administered. The apparent effects on milk composition are not immediately logical, given that the most widely known effect of Bovaer is reduced feed intake, only partially offset by lower milk yield – and in the present study, yield was generally unaffected. It would therefore be expected that the cow's energy status would decline when Bovaer is administered (possibly with mobilisation of body tissue), due to a presumed decrease in feed intake. This is normally expected to reduce the protein concentration and increase the concentration of 3-hydroxybutyrate in the milk – but the opposite appears to be the case in the analysed milk samples. In Danish university trials, an increase in the milk's fat and urea content has been documented as

as a result of feeding Bovaer (Maigaard et al., 2024; Kjeldsen et al., 2024; Johansen et al., 2025a; Johansen et al., 2025b), whilst the protein concentration in the trials has generally remained unaffected. These responses have so far been explained by changes in the cow's energy status resulting from reduced feed intake. The analyses of fat, protein and urea concentration in these university trials were carried out using FT-MIR scanning, on the same analytical platform as that used for production monitoring in practice.

The conflicting expectations and apparent effects on milk composition may be due to the fact that the analytical method used for milk is not robust against an unknown but altered metabolite profile in milk, which may be caused by Bovaer's effect on rumen fermentation (Rocchetti et al., 2026). Milk composition is predicted using FT-IR scanning, and it is expected that the calibration models have been optimised without access to a larger sample set from cows fed Bovaer. If feeding with Bovaer has a spectral signature not included in the calibration models used, this may result in unexpected deviations. It is noteworthy that by far the strongest Bovaer effect has been observed for 3-hydroxybutyrate, which is already known to be difficult to determine using the method employed, and it will often be the weakest calibration models that are most affected by spectral shifts. The present studies point to a possible need to address the analytical challenges that appear to be associated with the introduction of Bovaer into feed.

Health

In most herds, the administration of Bovaer had been discontinued by the time the project visited, and it has not been possible to contribute to the assessment of the negative effects on, for example, health, as reported by the farmers. In the present study, no effect of Bovaer was detected on digestive and metabolic disorders, mastitis or mortality in cows. One of the challenges in interpreting the available data is that the dosage of Bovaer has in many cases been reduced following the experience of negative effects of Bovaer. One possibility that cannot be ruled out in this study is that the herds have succeeded in limiting any adverse effects of Bovaer by reducing the dose in relation to the cows' tolerance. It is not possible to analyse retrospectively what the impact on production would have been if all herds had been fed a dose of 60 mg 3-NOP throughout the planned period.

Despite the limited impact of Bovaer on milk production and health statistics, this study cannot rule out the possibility that Bovaer may have a negative effect on production and health, but any such effect is difficult to describe retrospectively, as there are generally no good records of what ails cows that have 'dropped out' in reality. Furthermore, there is very little sample material available to describe the feeding regime in place whilst the negative effect of Bovaer may have been occurring. Furthermore, it is not known whether the reduction in the Bovaer dose has had a genuine mitigating effect, and/or whether supportive treatment of the cows has meant that any effect of Bovaer was dampened to such an extent that it could not be detected. In future studies on the effect of Bovaer on milk production, it would be advisable to monitor herds that are introducing Bovaer before adding it to the feed. If possible, herds should be fed a fixed ration composition for at least 14 days before Bovaer is added, and the ration should be kept unchanged for a period following the introduction of Bovaer. If this protocol is combined with the collection of feed samples before and during the introduction of Bovaer, as well as close monitoring of the cows' health and production, a significantly stronger foundation can be established for determining whether there are interactions between the ration composition and/or qualitative characteristics of the ration and any effects of Bovaer.

Feed intake

It is well documented that Bovaer can have a significant negative effect on feed intake, which has been observed, for example, in a number of Danish studies at Aarhus University (Lund et al., 2026), but also documented in a recent meta-analysis that utilises data from international trials (Martins et al., 2025). Very few of the participating herds carried out actual recordings of feed intake, and the vast majority of herds did not weigh leftover feed. There are a number of examples among the participating

herds experienced very large declines in feed intake following the introduction of Bovaer, and there are examples showing that this effect persists throughout the period during which Bovaer is administered. It would be highly desirable for future research on Bovaer to seek to understand which factors contribute to significant reductions in feed intake when Bovaer is administered in some herds. It is currently very uncertain which characteristics of the feed might cause the decline, and what the underlying physiological response is. It would also be desirable for herds to make an effort to document actual feed intake before, during and after the introduction of Bovaer by weighing both the fed ration and the feed residues, as well as documenting the ration's composition by taking samples of the mixed rations.

4. Conclusion

Analysis of data from 73 Danish dairy herds that fed the feed additive Bovaer in 2025 could not demonstrate any negative effects on milk production (measured as ECM yield), the somatic cell count in milk, treatments for mastitis, digestive and metabolic disorders, or cow mortality during periods of Bovaer administration, despite the fact that approximately two-thirds of the project hosts in this study reported varying degrees of negative experiences with the administration of Bovaer.

A significant number of herds used a reduced dosage of Bovaer during the period, and it is not possible to determine whether this adjustment of the dose mitigated any negative effects of Bovaer. A number of herds experienced significant reductions in feed intake when Bovaer was administered, but the study does not have a sufficient data basis to link a reduction in feed intake to generally described characteristics of the feed or the production system.

Feeding Bovaer had the greatest effect on milk analyses, particularly on the predicted concentrations of protein, urea and 3-hydroxybutyrate in milk. The study raises the question of whether it is the apparent predicted traits that are actually changing, or whether feeding with Bovaer introduces a bias because the calibration and validation basis for the FT-MIR models for protein, urea and 3-hydroxybutyrate do not include a sufficient number of milk samples from cows assigned to Bovaer.

The many different descriptive variables included in this study generally had very little explanatory power in describing the variation in herds' potential response to Bovaer. There is a need for more systematic studies with a better description of feeding and production conditions, alongside monitoring of production and health in dairy herds introducing Bovaer. In addition, efforts are needed to ensure the validity of the analyses used for production monitoring, particularly for milk.

5. References

- Bampidis, V., G. Azimonti, M. de Lourdes Bastos, H. Christensen, B. Dusemund, M. F. Durjava, M. Kouba, M. Lopez-Alonso, S. L. Puente, F. Marcon, B. Mayo, A. Pechova, M. Petkova, F. Ramos, Y. Sanz, R. E. Villa, R. Woutersen, G. Aquilina, G. Bories, P. G. Brantom, J. Gropp, K. Svensson, L. Tosti, M. Anguita, J. Galobart, P. Manini, J. Tarres-Call, and F. Pizzo. 2021. Safety and efficacy of a feed additive consisting of 3-nitrooxypropanol (Bovaer® 10) for ruminants for milk production and reproduction (DSM Nutritional Products Ltd). *EFSA Journal* 19:6905. <https://10.2903/j.efsa.2021.6905>
- Grelet, C., C. Bastin, M. Gelé, J.-B. Davière, M. Johan, A. Werner, R. Reding, J. A. Fernandez Pierna, F. G. Colinet, P. Dardenne, N. Gengler, H. Soyeurt, and F. Dehareng. 2016. Development of Fourier transform mid-infrared calibrations to predict acetone, beta-hydroxybutyrate, and citrate contents in bovine milk through a European dairy network. *J. Dairy Sci* 99:4816-4825. <http://dx.doi.org/10.3168/jds.2015-10477>
- Hansen, P. W. 1998. Spectroscopic Analyses on Dairy Products. Pages 126. Ph.D. Thesis. The Royal Veterinary and Agricultural University, Copenhagen
- Harmon, R. J. 1994. Physiology of mastitis and factors affecting somatic cell counts. *J. Dairy Sci* 77:2103-2112. [https://10.3168/jds.S0022-0302\(94\)77153-8](https://10.3168/jds.S0022-0302(94)77153-8)
- Humphreys, D. J. 1988. *Veterinary Toxicology*. 3rd ed. Baillière Tindall, London, England.
- Johansen, M., M. Maigaard & P. Lund. 2025a. Effect of Bovaer inclusion in diets with a high proportion of grass-clover silage of varying nutritional quality on gas emissions and production performance in dairy cows. *Journal of Dairy Sci*, 108:4975–4987. <https://doi.org/10.3168/jds.2024-25949>
- Johansen M., Maigaard, M. & Lund, P. 2025b. Effect of Bovaer inclusion in rations with a high proportion of maize silage harvested at different stubble heights on production performance and gas emissions in dairy cows. *Animal Feed Sci and Tech*, 329:116512. <https://doi.org/10.1016/j.anifeedsci.2025.116512>
- Kjeldsen, M. H., M. R. Weisbjerg, M. Larsen, O. Højberg, C. Ohlsson, N. Walker, A. L. F. Hellwing, and P. Lund. 2024. Gas exchange, rumen hydrogen sinks, and nutrient digestibility and metabolism in lactating dairy cows fed 3-nitrooxypropanol and cracked rapeseed. *J. Dairy Sci*. 107:2047–2065. <https://doi.org/10.3168/jds.2023-23743>.
- Lund, P., M. H. Kjeldsen, M. Johansen, M. Maigaard, S. Lashkari, C. F. Børsting, and M. B. Jensen. 2026. Overview of trials with Bovaer for dairy cows conducted at the Department of Animal and Veterinary Sciences, Aarhus University. Advisory note from DCA – National Centre for Food and Agriculture, Aarhus University. 9 pages. Submitted: 16 January 2026.
- Maigaard, M., M. R. Weisbjerg, M. Johansen, N. Walker, C. Ohlsson & P. Lund. 2024. Effects of dietary fat, nitrate, and 3-NOP and their combinations on methane emission, feed intake and milk production in dairy cows. *Journal of Dairy Sci*, 107:220-241. <https://doi.org/10.3168/jds.2023-23420>
- Martins, L. F., M. Maigaard, M. Johansen, P. Lund, X. Ma, M. Niu & A. N. Hristov. 2025. Lactational performance effects of 3-nitrooxypropanol supplementation to dairy cows: A meta-regression. *Journal of Dairy Sci*, 108:1538-1553. <https://doi.org/10.3168/jds.2024-25653>.
- Nielsen, L. A. H., N. I. Nielsen, M. V. Byskov, F. H. Lau-Jensen, and A. Fogh. 2025. Dairy farmers' experiences with the use of Bova. SEGES Innovation P/S, Aarhus N, Denmark. [Landbrugsinfo 16 December 2025, updated 10 April 2026.](https://www.landbrugsinfo.dk/public/6/5/5/foder_fodring_malkeproducenters_oplevelser_med_brug_af_bovaer)
https://www.landbrugsinfo.dk/public/6/5/5/foder_fodring_malkeproducenters_oplevelser_med_brug_af_bovaer

- Rocchetti, G., F. Froidi, M. Lapris, M. Moschini, P. Bani, L. Cattaneo, and E. Trevisi. 2026. A data fusion approach reveals the effects of 3-nitrooxypropanol on the rumen fluid and milk metabolomes of lactating Holstein dairy cows. *J. Dairy Sci* 109:2714-2726. <https://doi.org/10.3168/jds.2025-26969>
- Sjaunja, L. O., L. Baevre, L. Junkkarinen, J. Pedersen, and J. Setälä. 1991. A Nordic proposal for an energy-corrected milk (ECM) formula. Pages 156–157 in *EAAP Publication 50*. Centre for Agricultural Publishing and Documentation (PUDOC), Wageningen, The Netherlands.
- Wilmink, J. B. M. 1987. Adjustment of test-day milk, fat and protein yield for age, season and stage of lactation. *Livest. Prod. Sci* 16:335–348. [https://doi.org/10.1016/0301-6226\(87\)90003-0](https://doi.org/10.1016/0301-6226(87)90003-0)