



# Effect of diets with different crude protein levels on ammonia and greenhouse gas emissions from a naturally ventilated dairy housing

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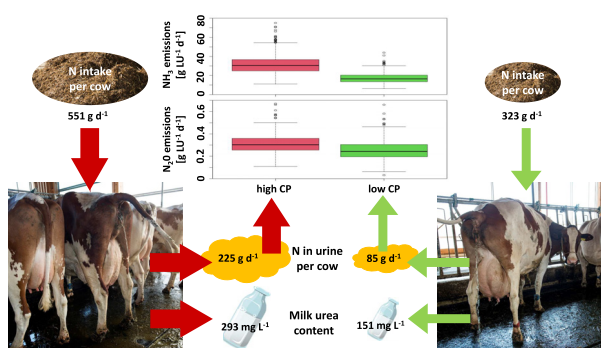
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## HIGHLIGHTS

- Emissions from two herds fed different diets were compared on a practical scale.
- Lower dietary crude protein markedly reduced ammonia and nitrous oxide emissions.
- Dietary protein, urinary nitrogen excretion and milk urea content show relations.
- Higher temperature and wind speed increased ammonia and nitrous oxide emissions.
- Temperature and wind speed affected ammonia emissions more at higher protein level.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Less crude protein (CP) in the diet can reduce nitrogen excretion of dairy cattle and lower their ammonia (NH<sub>3</sub>) and nitrous oxide (N<sub>2</sub>O) formation potential. The diet composition might also affect emissions of methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>). However, previous studies did not investigate the effect of diets with different CP levels that are customary practice in Switzerland on NH<sub>3</sub> and greenhouse gas emissions on a practical scale. In a case-control approach, we quantified the emissions (NH<sub>3</sub>, N<sub>2</sub>O, CH<sub>4</sub>, CO<sub>2</sub>) in two separate but identical compartments of a naturally ventilated cubicle housing for lactating dairy cows over six days by using a tracer ratio method. Cows in one compartment received a diet with 116 g CP per kilogram dry matter (DM), in the other compartment with 166 g CP kg<sup>-1</sup> DM. Subsequently, diets were switched for a second 6-day measurement phase. The results showed that the diet, aside from outside temperature and wind speed in the housing, was driving NH<sub>3</sub> and N<sub>2</sub>O emissions. NH<sub>3</sub> and N<sub>2</sub>O emission reduction per livestock unit (LU) was on average 46 % and almost 20 %, respectively, for the diet with low CP level compared to the higher CP level. In addition, strong relationships were observed between the CP content of the diet, N excretion in the urine and the milk urea content. An increased temperature or wind speed led to a clear increase in NH<sub>3</sub> emissions. Differences in CH<sub>4</sub> and CO<sub>2</sub> emissions per LU indicated a significant influence of the diet, which cannot be attributed to the CP content. Our herd-level study demonstrated that a significant reduction in NH<sub>3</sub> and N<sub>2</sub>O emissions related to LU, energy-corrected milk as well as DM intake can be achieved by lowering the CP content in the diet.

## 1. Introduction

The adverse effects of ammonia (NH<sub>3</sub>) emissions on ecosystems include acidification and eutrophication of soils and freshwaters, which leads to increased productivity and a reduction in biodiversity (Sutton et al., 2011). In addition, NH<sub>3</sub> plays vital roles in the formation of secondary aerosols

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associated with increased risks of respiratory diseases in humans. For instance, according to models by Backes et al. (2016), a 50 % reduction in NH<sub>3</sub> emissions leads to a 24 % reduction in particulate matter 2.5 concentrations in north-western Europe. Methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) are aside from carbon dioxide (CO<sub>2</sub>) the most potent anthropogenically emitted greenhouse gases (GHG), strong air pollutants and stratospheric ozone depleting substances, both with showing a strong increase in recent years (WMO, 2019). In Switzerland 94 % of total NH<sub>3</sub> emissions in 2019 originated from agriculture, predominantly (93 %) from livestock farming (Kupper et al., 2022). In terms of GHG, in 2020 around 64 % of N<sub>2</sub>O emissions and 83 % of CH<sub>4</sub> emissions originated from agricultural sources (FOEN, 2022). Reduction targets for NH<sub>3</sub> emissions have set internationally. For example, the reduction targets for the countries of the European Union are set out in the National Emission reduction Commitments Directive (European Union, 2016). Compared to the EU countries, the Swiss agricultural sector has defined quite high targets of around 40 % reduction in NH<sub>3</sub> emissions (FOEN and FOAG, 2008). At the COP21 Conference of Parties (UNEP, 2015), it was agreed that countries must make determined contributions and commitments to reduce national GHG emissions. Owing to the high non-CO<sub>2</sub> GHG emissions of the agricultural sector, one goal of the Swiss agricultural climate strategy has been to reduce agricultural emissions of these gases by at least one third by 2050 as compared with 1990 (FOAG, 2011; Wiedemar and Felder, 2011).

To achieve these challenging goals, emission mitigation measures must be developed and implemented comprehensively. Feeding strategies are 'begin-of-pipe' measures, which address the root cause of emissions. Another advantage is that no structural and technical changes are required and therefore implementation can be realised rather quickly on a large number of farms. Feeding has a considerable influence on the nitrogen (N) excretion of ruminants and thus also on gaseous N losses (Bittman et al., 2014). An oversupply with crude protein (CP) leads to increased urinary N excretion (Castillo et al., 2001; Kebreab et al., 2002). Numerous studies have shown with modelling, on a laboratory or practical scale, that at lower dietary CP content, urinary N excretion and thus NH<sub>3</sub> emissions are reduced (e.g. De Boer et al., 2002; Edouard et al., 2019; Powell et al., 2008, 2011; Smits et al., 1995; Todd et al., 2006; Van Duinkerken et al., 2005, 2011). Dijkstra et al. (2013) concluded in a literature review that aside from NH<sub>3</sub>, N<sub>2</sub>O emissions from urine and manure deposited in housings are controlled by N excretion of cattle and which, in turn by the CP content in the diet. N<sub>2</sub>O is mainly generated as a by-product of enhanced microbial N conversion, ammonium oxidation and incomplete denitrification (Butterbach-Bahl et al., 2013). In Switzerland, however, CP-reduced feeding has barely been implemented in dairy farming, in contrast to pig fattening (Wasem and Probst, 2022), but possibilities of CP-optimised feeding strategies for dairy cows and their implementation are being discussed (FOAG, 2016; FOAG, 2022).

We hypothesise that diets containing less CP will have significantly lower housing-based NH<sub>3</sub> and N<sub>2</sub>O emissions compared to those containing more CP. Thus, the objectives of our study were (i) to quantify the NH<sub>3</sub> and N<sub>2</sub>O mitigation potential of a diet with low CP content, as compared with high CP, in a naturally ventilated cubicle housing, whereby both diets reflect practical conditions in Switzerland; (ii) to measure the CH<sub>4</sub> and CO<sub>2</sub> emissions of the investigated diets with regard to trade-off effects; (iii) to identify other potentially influencing variables on the emissions, namely air temperature and wind speed; and (iv) to further characterise animal performance parameters (e.g. milk yield and contents, live weight, feed intake).

## 2. Materials and methods

### 2.1. Housing and animals

The experiment was conducted in February and March 2017 in the experimental dairy housing for emission measurements in north-eastern Switzerland (47°49'N, 8°92'E). The naturally ventilated housing consisted of two identical, spatially separated compartments, each for 20 lactating

cows and equipped with three cubicle rows with straw mattresses (Mohn et al., 2018; Poteko et al., 2020). The solid floors were designed without slopes and covered with rubber mats. Manure removal and ventilation management were the same in both compartments. The aisles were cleaned 12 times per day with stationary scrapers. The longitudinal facades equipped with flexible curtains were closed, whereas spaced boards in the upper area enabled sufficient ventilation of the housing. During the investigations, the cows did not have access to the outdoor exercise area.

In both compartments, the dairy herd consisted of 20 primiparous and multiparous lactating cows of Brown Swiss and Swiss Fleckvieh breeds. The herds were balanced as best as possible for breed, live weight, lactation number, days in milk and milk yield. The cows were milked twice a day at 05:30 and 16:30 in the milking parlour, which was located in the centre of the housing between the two compartments.

The experimental protocol complied with the Swiss legislation on animal welfare and was approved (ZH091/15) by the Cantonal Veterinary Office of Zürich prior to the start of the investigations.

### 2.2. Feeding and experimental design

Two dairy diets with different CP content were fed. Both diets were made of components typical for the region and consisted of a mixed ration based on grass silage, maize silage, hay, and sugar beet pulp silage (Table 1). Details on the feed analysis are provided in Table 1. In terms of energy, both base diets were calculated for slightly >29 kg milk per day. New portions of the mixed rations provided with ad libitum access were offered at 16:45 after milking. Two different concentrates, one rich in energy and one rich in protein, were fed individually according to milk yield and body condition score by automatic feeder. The cows had permanent access to water.

The measurements in the two experimental compartments were done at the same time, ensuring comparable climatic conditions. To determine the effect of the two feeding strategies on emissions by taking into account the effects of herds and housing compartment, a crossover design was chosen. The measurement periods lasted six consecutive days each. In the first measuring period (22 to 28 February 2017), the cows in the northern compartment were fed the diet with the low CP content and at the same time the cows in the southern compartment were fed the diet with the high CP content. Subsequently, in the second measurement period (10 to 16 March 2017), the diets were switched. To habituate the animals and in particular the ruminal microbial composition to the respective diet, each measurement was preceded by a 10-day adaptation phase.

**Table 1**  
Ingredients and nutrient composition of the diets with lower crude protein (CP) level ('Low CP') and higher CP level ('High CP').

Item	Low CP	High CP
Averaged relative proportions of ingredients in % in dry matter (contents: CP in g kg <sup>-1</sup> of dry matter; net energy for lactation NEL in MJ kg <sup>-1</sup> of dry matter)		
Grass silage	24.2 (119; 5.1)	43.4 (187; 6.0)
Maize silage	28.0 (76; 6.8)	26.2 (76; 6.8)
Hay	26.2 (114; 5.7)	11.0 (114; 5.7)
Sugar beet pulp silage	6.3 (98; 7.1)	1.9 (98; 7.1)
Mineral feed	0.6	0.5
Salt	0.2	0.2
Urea	0	0.6 (2736; 0)
Dextrose	2.7 (0; 8.3)	0
Concentrate (feed mix wagon)	6.4 (504; 7.7)	10.0 (504; 7.7)
Concentrate rich in protein (automatic feeder)	0	0.5 (440; 8.3)
Concentrate rich in energy (automatic feeder)	5.7 (198; 8.5)	5.7 (198; 8.5)
Nutrients of diet (means per treatment)		
Crude protein CP (g kg <sup>-1</sup> of dry matter)	115	169
Crude fibre CF (g kg <sup>-1</sup> of dry matter)	239	195
Neutral detergent fibre NDF (g kg <sup>-1</sup> of dry matter)	493	398
Acid detergent fibre ADF (g kg <sup>-1</sup> of dry matter)	280	227
Net energy for lactation NEL (MJ kg <sup>-1</sup> of dry matter)	6.3	6.4

### 2.3. Emission measurements

To calculate emissions from the described naturally ventilated housing compartments with dynamic airflows, a dual tracer ratio method with artificial tracer gases was used (Mohn et al., 2018; Poteko et al., 2020; Schrade et al., 2012). The diluted tracer gases (concentration around 2000 ppm  $\pm$  2 %; Carbagas AG, Gümliigen, Switzerland) sulphur hexafluoride (SF<sub>6</sub>) and trifluoromethyl sulphur pentafluoride (SF<sub>5</sub>CF<sub>3</sub>) were dosed continuously via a steel tubing system with critical capillaries (hole diameter 30  $\mu$ m; Lenox Laser, Inc., Glen Arm, MD, USA) near the ground on both sides of the aisles to mimic the main emission sources such as soiled exercise areas and animals. Different tracer gases (SF<sub>6</sub>, SF<sub>5</sub>CF<sub>3</sub>) were used in both compartments to detect any cross-contamination between compartments during simultaneous measurements. A sampling system consisting of 24 critical glass capillaries (hole diameter 250  $\mu$ m; Thermo Instruments GmbH, Dortmund, Germany, and LouwersHanique B.V., Hapert, The Netherlands) protected with polytetrafluoroethylene (PTFE) membrane filters (TE 38, 37 mm, Whatman plc, Maidstone, United Kingdom) installed at a height of 2.5 m connected by PTFE tubes ensured representative and integrative air sampling for each compartment. The air sample for background concentration measurements of N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub> was collected approx. 30 m south of the housing analogous to the in-house measurement with a PTFE tube with critical glass capillaries. The NH<sub>3</sub> background concentration was measured with passive samplers (radiello®, Forschungsstelle für Umweltbeobachtung AG, Rapperswil, Switzerland), integrating over individual measuring periods, about 80 m north of the housing.

NH<sub>3</sub>, CH<sub>4</sub> and CO<sub>2</sub> concentrations were analysed with cavity ring-down spectroscopy (NH<sub>3</sub>: G2103; CH<sub>4</sub>, CO<sub>2</sub>: G2301; Picarro, Inc., Santa Clara, CA, USA). N<sub>2</sub>O concentration was measured using a quantum cascade laser absorption spectrometer (QCLAS, QC-TILDAS-CS, Aerodyne Research, Inc., Billerica, MA, USA) after drying the sample gas with a Nafion™ permeation device (PD-100 T-72, Perma Pure LLC, Lakewood, NJ, USA). Concentrations of SF<sub>6</sub> and SF<sub>5</sub>CF<sub>3</sub> were analysed by gas chromatography with electron capture detection (GC-ECD, 7890A, Agilent Technologies AG, Basel, Switzerland).

The emission calculation was based on the assumption that the ratio of the background-corrected concentrations of the respective emitted target gas  $c_G$  and the dosed artificial tracer gas  $c_T$  corresponds to the ratio of their mass flows ( $\dot{m}$ ). Thus, the emissions can be calculated with the following equation:

$$\dot{m}_G = \frac{\dot{m}_T \cdot c_G}{c_T}$$

The applied quasi-continuous measurement sequence with alternating sampling of the two compartments combined with periodic sampling of the background, the centre section and calibrations provided emission data of at least two 10-min measuring phases per compartment and hour. CH<sub>4</sub> and CO<sub>2</sub> emission data during milking times were excluded from further analysis because none or only a part of the herds was in the compartments during these times. Details on gas analysis and the dual tracer ratio method as well as its validation are described in Mohn et al. (2018).

### 2.4. Climate data

In the middle of each compartment at a height of 2.5 m, wind data were measured using 3D ultrasonic anemometers (Ultra-sonic Anemometer 3D, Adolf Thies GmbH & Co. KG, Göttingen, Germany). The outdoor climate was recorded 60 m to the south-west of the housing by using the same measuring devices, whereby the wind data were measured at a height of 10 m and the air temperature at a height of 2.5 m.

### 2.5. Sampling and collection of animal-related data

The masses of the mixed ration offered and of the leftovers in both compartments were weighed and documented daily. Concentrates'

consumption was recorded at individual animal level by the herd management software program (Fullexpert, Fullwood, Ellesmere, United Kingdom). Samples of the mixed rations in the feed mix wagon and of the leftovers each per herd were collected daily, dried at 60 °C for 48 h and pooled in proportion to the amount in dry matter of feed intake or of the leftovers per measuring period. In addition, samples of all feed ingredients were taken once per measuring period. Dry matter was obtained by drying the samples at 105 °C for 24 h. Eating and ruminating activity of 10 focus cows of each herd were determined on an hourly basis with noseband pressure sensors of the RumiWatch System using the converter V0.7.4.5 (Itin + Hoch GmbH, Liestal, Switzerland) as described by Steinmetz et al. (2020). For further evaluation, mean daily values per treatment were formed.

Milk yield at individual animal level was recorded at each milking time by using a milk meter (EasyFlow, Fullwood, Ellesmere, United Kingdom). Furthermore, milk samples were obtained individually at morning and evening milking times every second day during the measuring period. In parallel, pool samples per herd were prepared according to the milk yield of individual animals. All milk samples were preserved by adding bronopol (D&F, Inc., Dublin, CA, USA).

Spot urine samples (spontaneous urinations respective by stimulating the area below the vulva) of nine focus cows per herd, three in each lactation stage, were collected twice per measuring period around the morning milking time and frozen.

The live weight of individual animals was determined by measuring the girth with a measuring tape before and after each measurement period, and mean values were calculated from these data.

### 2.6. Laboratory analysis and calculations

The dried feed and leftover samples were ground using a 1.0-mm screen (Brabender mill with titanium blades; Brabender GmbH & Co. KG, Duisburg, Germany). The laboratory dry matter and ash contents of feed items were determined by drying for 3 h at 105 °C and incineration at 550 °C (prepASH 340, Precisa, Dietikon, Switzerland) until a constant mass was reached. The N, acid detergent fibre (ADF) and neutral detergent fibre (NDF) contents of grass silage, maize silage and hay were determined by near infrared spectroscopy (Ampuero Kragten and Wyss, 2014). The total N content of the mixed rations, concentrates, sugar beet pulp silage and leftovers was analysed by the Dumas method (ISO, 2008b; method 16,634–1) and that of urine by using the Kjeldahl method (AOAC International, 1995; method 988.05). To calculate the CP content of feeds and leftovers, the results were multiplied by 6.25, according to a protein content of about 16 %. NDF and ADF contents (method ISO 16472, 2006 for NDF and ISO 13906, 2008a for ADF) of mixed rations, concentrates, sugar beet pulp silage and leftovers were determined using a FIBREITHERM® (Gerhardt, Königswinter, Germany). Net energy for lactation content of grass silage, maize silage, hay and sugar beet pulp silage was calculated according to Agroscope (2017).

The bronopol-conserved milk samples of individual animals and pool samples, according to milk yield per herd, were analysed using Fourier-transform mid-infrared spectroscopy (MilkoScan FT6000, Foss Electric, Hillerød, Denmark) at the Swiss routine milk analysis laboratory (SuisseLab AG, Zollikofen, Switzerland) to determine fat, protein and urea contents. The energy-corrected milk (ECM) yield was calculated using the aliquot values of the individual animals on a daily basis. For further statistical analysis, daily means per treatment were used.

Creatinine analysis in urine was performed using a commercial kit (Biotechnica Instruments S.p.A., Roma, Italy), following manufacturer instructions with an autoanalyser (BT1500, Biotechnica Instruments S.p.A., Roma, Italy). Urine volume was calculated individually according to Burgos et al. (2005) by using creatinine concentration in urine and live weight.

### 2.7. Statistical analysis

Statistical analyses were performed using the statistical software platform R version 4.2.2. The statistical evaluation of the NH<sub>3</sub>, CH<sub>4</sub>, CO<sub>2</sub> and

$N_2O$  emissions was done on the time basis of 10-min measurement intervals. Analogously, for the temperature and wind data with a higher temporal resolution, mean values of the respective 10-min measurement intervals were formed. Linear mixed-effects models (Pinheiro and Bates, 2000) were used to describe the emissions. These models took into account the measurement day as a random effect. The models were selected from several models constructed on the basis of sets of weakly correlated variables, whereas Akaike and Bayesian information criteria served as selection criteria. In the  $NH_3$  emission models, the target variable  $NH_3$  (per livestock unit [LU] in  $g\ LU^{-1}\ d^{-1}$ ; per dry matter intake (DMI) in  $g\ kg^{-1}\ DMI^{-1}$ ; per ECM in  $g\ kg^{-1}\ ECM^{-1}$ ) was logarithmically transformed to fulfil the model requirements of the normal distribution and afterwards transformed back. No transformation was necessary for the statistical analysis of the other target variables ( $N_2O$ ,  $CH_4$ ,  $CO_2$ ). The fixed effects for both target variables,  $NH_3$  and  $N_2O$ , were the diet, the outside air temperature and the wind speed inside the housing. For models of the other two target variables, namely  $CH_4$  and  $CO_2$  emissions (in  $g\ LU^{-1}\ d^{-1}$ ), the fixed effect was the diet. Graphical residual analyses were used to check the models' assumptions. At  $p < 0.05$ , the effects were considered statistically significant.

### 3. Results

#### 3.1. Effect on performance, feed intake, N excretion, feeding and ruminating behaviour

There was no difference in live weight of the cows in both treatments (Table 2). The ECM yield was markedly higher for the diet with higher CP level. The feed intake in dry matter (the DMI) in the treatment with high CP level was about 16 % higher than in the treatment with low CP level and was accompanied by a numerically slightly longer feeding time. Rumination time was longer at the low CP level than at the high CP level. The mean N intake per cow was about 70 % higher in the high CP than in the low CP treatment. The milk urea content was almost twice as high in cows fed with the diet with high CP level as in cows fed with the diet with low CP level, and the urinary N excretion was 2.7 times greater at high CP level. Fig. 1 shows the relationship between milk urea content and urinary N excretion based on data from the individual urine sampling of focus animals. The two treatments low CP and high CP are clearly visible as separated clusters.

#### 3.2. $NH_3$ emissions

With the applied statistical models, the diet ( $p < 0.001$ ), the outside air temperature ( $p < 0.001$ ) and the wind speed inside the housing ( $p < 0.001$ ) were identified as variables driving  $NH_3$  emissions. The treatment with high CP level in the diet resulted on average in almost twice as high  $NH_3$  emissions with  $31.9\ g\ LU^{-1}\ d^{-1}$  compared with the treatment with low CP level with  $17.3\ g\ LU^{-1}\ d^{-1}$ . The influences of the diet, the outside air temperature and the wind speed inside the housing on  $NH_3$  emissions

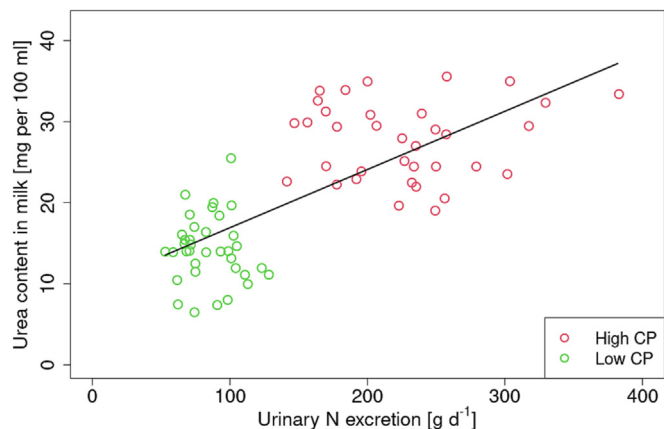
**Table 2**

Performance, feed intake, eating and ruminating behaviour in both treatments, low crude protein (Low CP) and high crude protein (High CP) level in the diet.

Item	Low CP	High CP
Live weight per cow (kg)	714 ± 85	719 ± 83
Dry matter intake DMI per cow ( $kg\ d^{-1}$ )	17.6 ± 0.3	20.4 ± 1.0
N intake per cow ( $g\ d^{-1}$ )	323 ± 5.7	551 ± 26
Energy-corrected milk ECM yield per cow ( $kg\ d^{-1}$ )	24.8 ± 0.8	27.8 ± 2.3
Milk urea content ( $mg\ L^{-1}$ )	151 ± 21	293 ± 27
N excreted in urine per cow ( $g\ d^{-1}$ ) <sup>a</sup>	84.8 ± 19.0	224.9 ± 56.3
Ruminating time per cow ( $min\ d^{-1}$ ) <sup>b</sup>	553 ± 14	535 ± 23
Eating time per cow ( $min\ d^{-1}$ ) <sup>b</sup>	441 ± 17	454 ± 17

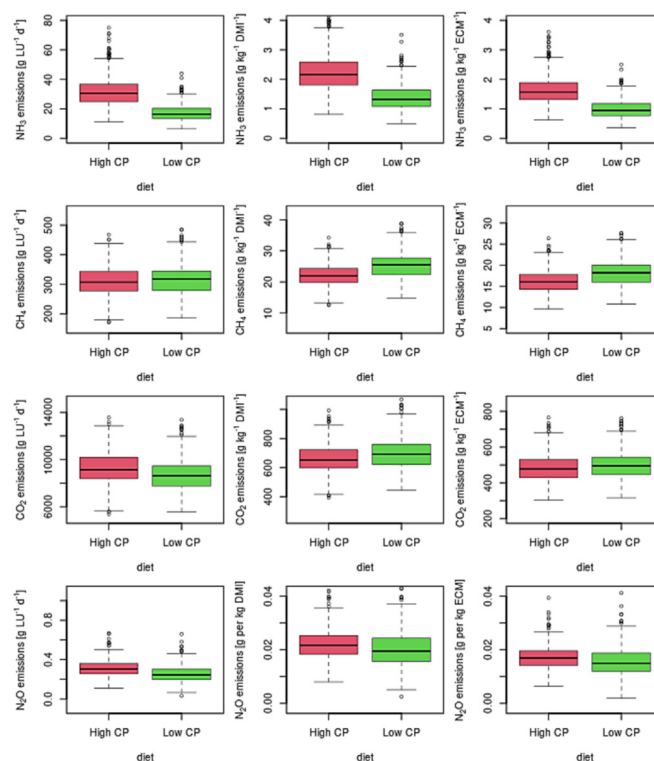
<sup>a</sup> Spot sampling two times per measuring phase in the morning, nine focus animals per treatment; estimation of urine volume via creatinine concentration in urine (Burgos et al., 2005).

<sup>b</sup> Ten focus cows per treatment.



**Fig. 1.** Relationship between urea content in milk (mg per 100 ml) and urinary N excretion ( $g\ d^{-1}$ ) per cow in treatments with high or low crude protein (CP) level in the diet.

were highly significant ( $p < 0.001$ ) regardless of whether emissions were related to LU, to DMI or to ECM. While  $NH_3$  emissions per LU were 1.8 times higher at the high CP than at the low CP level in the diet, the factor for ECM and DMI was 1.6 (Fig. 2). The outside air temperature over both measurement periods was on average  $6\ ^\circ C$ , and individual 10-min values covered a large range from  $-3$  to  $19\ ^\circ C$ .  $NH_3$  emissions increased with rising temperature. This relationship was most evident for the treatment with the high CP level (Fig. 3). The effect of wind speed inside the housing on  $NH_3$  emission is shown in Fig. 4. A clear increase in  $NH_3$  emissions with increasing wind speed was observed, for wind speeds in both compartments between around 0 and  $0.5\ m\ s^{-1}$ . As found for the effect of temperature, the effect of wind speed was more pronounced for the treatment with high CP level.



**Fig. 2.**  $NH_3$ ,  $CH_4$ ,  $CO_2$  and  $N_2O$  emissions by diet (High CP, Low CP) related to different reference variables: per LU ( $g\ LU^{-1}\ d^{-1}$ ), per DMI ( $g\ kg^{-1}\ DMI^{-1}$ ) or per ECM ( $g\ kg^{-1}\ ECM^{-1}$ ).



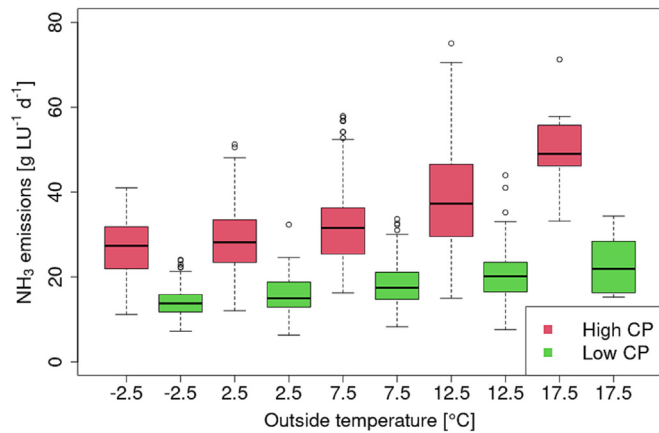


Fig. 3.  $\text{NH}_3$  emissions ( $\text{g LU}^{-1} \text{d}^{-1}$ ) by temperature classes ('-2.5':  $-5.0$  to  $0.0$  °C; '2.5':  $>0.0$  to  $5.0$  °C; '7.5':  $>5.0$  to  $10.0$  °C; '12.5':  $>10.0$  to  $15.0$  °C; '17.5':  $>15.0$  to  $20.0$  °C) differentiated according to the treatments Low CP (green) and High CP (red) level in the diet.

### 3.3. Greenhouse gas emissions

$\text{CH}_4$  emissions ( $\text{g LU}^{-1} \text{d}^{-1}$ ) were significantly lower with high CP level in the diet ( $p = 0.024$ ) than with low CP level in the diet. Numerically, the difference between the average emission values of both treatments was small with  $310 \text{ g LU}^{-1} \text{d}^{-1}$  at high CP level and  $316 \text{ g LU}^{-1} \text{d}^{-1}$  at low CP level. Fig. 2 shows that the differences in  $\text{CH}_4$  emissions per DMI ( $\text{g kg}^{-1} \text{DMI}^{-1}$ ) and per ECM ( $\text{g kg}^{-1} \text{ECM}^{-1}$ ) were even more evident.

Analogous to  $\text{NH}_3$  emissions, the statistical model identified the diet ( $p < 0.001$ ), the outside air temperature ( $p < 0.001$ ) and the wind speed inside the housing ( $p < 0.001$ ) as factors promoting  $\text{N}_2\text{O}$  emissions. The mean  $\text{N}_2\text{O}$  emission per LU was nearly 20 % lower at low CP level ( $0.25 \text{ g LU}^{-1} \text{d}^{-1}$ ) than at high CP level in the diet ( $0.31 \text{ g LU}^{-1} \text{d}^{-1}$ ). The difference between the two diets was less pronounced when  $\text{N}_2\text{O}$  emissions were related to DMI and ECM. The effect of the outside air temperature and wind speed in the housing on  $\text{N}_2\text{O}$  emission per LU also was less evident compared with  $\text{NH}_3$  emissions.

Diet was identified as the sole factor ( $p < 0.001$ ) driving  $\text{CO}_2$  emission per LU ( $\text{g LU}^{-1} \text{d}^{-1}$ ). The boxplots in Fig. 2 indicate that  $\text{CO}_2$  emissions expressed per LU were markedly higher in the treatment with high CP level in the diet than in the treatment with low CP level. However, when  $\text{CO}_2$  emissions were related to DMI or ECM,  $\text{CO}_2$  emissions for the low CP level treatment were higher.

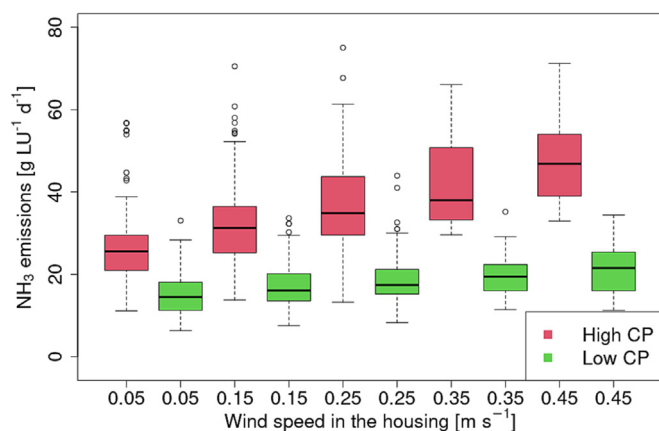


Fig. 4.  $\text{NH}_3$  emissions ( $\text{g LU}^{-1} \text{d}^{-1}$ ) by wind speed class inside the housing ('0.05':  $0.0$  to  $0.1 \text{ m s}^{-1}$ ; '0.15':  $>0.1$  to  $0.2 \text{ m s}^{-1}$ ; '0.25':  $>0.2$  to  $0.3 \text{ m s}^{-1}$ ; '0.35':  $>0.3$  to  $0.4 \text{ m s}^{-1}$ ; '0.45':  $>0.4$  to  $0.5 \text{ m s}^{-1}$ ) differentiated according to the treatments Low CP (green) and High CP (red) level in the diet.

## 4. Discussion

### 4.1. Relation between CP level, urinary N excretion and milk urea content

The average milk urea contents of our study with  $151 \pm 21 \text{ mg L}^{-1}$  at low CP level and  $293 \pm 27 \text{ mg L}^{-1}$  at high CP level are in the range of values observed in surveys on commercial farms in Switzerland (Schrade et al., 2012; Wasem and Probst, 2022) as well as recommendations with  $170\text{--}260 \text{ mg L}^{-1}$  (Bracher, 2011) and  $150\text{--}250 \text{ mg L}^{-1}$  (DLG, 2020). Differences in the CP level of the two diets were clearly reflected in the milk urea content and in the N excretion via urine (see Fig. 1) on both individual animal level and on treatment level (see Table 2). Correlations between N excretion in urine and milk urea content were also shown in a larger dataset collected for analogous experiments at Agroscope (Decker et al., 2021) and in several other studies and model calculations (e.g. Kauffman and St-Pierre, 2001; Nousiainen et al., 2004; Spek et al., 2013; Zhai et al., 2005). Therefore, milk urea content can be used as an indicator of urinary N excretion.

### 4.2. Effects of the diets on DMI, ECM, eating and ruminating behaviour

The higher DMI of dairy cows fed with the diet with high CP level was accompanied by an increased eating time compared with cows fed with the diet with low CP level. For the diet with low CP level, the lower DMI was reflected in shorter eating times. On the other hand, the rumination time was longer for cows offered the diet with low CP level. The reason could be the higher crude fibre content of the diet. In two French studies, the CP content of the two compared dairy cattle diets was around 12 % and around 18 % in dry matter (Edouard et al., 2016, 2019) and thus in a similar range as in the present study with around 12 % and 17 %. In both studies, the authors observed a higher DMI for the diet with a higher CP level, but differences were less pronounced than in our results. In the study by Broderick (2003), the increase in CP content at three different levels of 15.1 %, 16.7 % and 18.4 % was also accompanied by a slight increase in DMI. In contrast, the DMI was significantly lower for dairy cows fed a grass-based diet with a CP content around 18 % than for those fed a mixed diet with a CP content around 15 % (Almeida et al., 2022). Inconsistent results were observed in a study with five CP levels ranging from 13.5 % to 19.4 %, where the DMI of dairy cows did not differ significantly (Olmos Colmenero and Broderick, 2006). This finding was confirmed in a study by Broderick et al. (2015), who registered no effect on DMI for dairy cows offered diets with different CP levels of 15 % and 17 %.

In our study a higher ECM yield was detected for dairy cows fed the diet with high CP level. This result is in line with two French studies, mentioned above, which show a similar but less pronounced trend (Edouard et al., 2016, 2019). The lower ECM yield in our study is probably due to the markedly lower DMI of the animals. It is therefore difficult to derive general conclusions about the relationship between CP content of the diet and ECM from this study, as the diets differed not only in CP content but also in crude fibre content and DMI.

There is a need for research in this area, particularly in the investigation and optimisation of roughage-based diets as well as diets with significant amounts of fresh grass or grazing.

### 4.3. $\text{NH}_3$ emission

The observed range of  $\text{NH}_3$  emission values (10-min values) in the presented study was rather wide,  $7\text{--}75 \text{ g LU}^{-1} \text{d}^{-1}$ , compared with other winter measurements, comprised in a meta-analysis by Poteko et al. (2019), which are mostly temporally aggregated values, e.g. daily mean values. For example, in winter measurements in four Swiss commercial dairy housings,  $\text{NH}_3$  emissions in the range of 4 to  $35 \text{ g LU}^{-1} \text{d}^{-1}$  were detected for 36-min or 50-min averaged values (Schrade et al., 2012). Higher daily mean  $\text{NH}_3$  emission values, with around  $85 \text{ g LU}^{-1} \text{d}^{-1}$  were only determined in a one-day winter measurement by Snell et al. (2003). The very high  $\text{NH}_3$  emission values in our study were mainly driven by a co-

occurrence of high temperatures in the variant with high CP content in the diet (see Fig. 3). Temperature affects several sub-processes during  $\text{NH}_3$  formation and release such as urease activity, dissociation equilibrium and liquid to gas transfer (Sommer et al., 2006). A temperature or seasonal effect on  $\text{NH}_3$  emission in cubicle housings for dairy cattle has been detected in numerous studies (e.g. Hempel et al., 2016; Ngwabie et al., 2011; Schrade et al., 2012) and reported in meta-analyses (e.g. Bougouin et al., 2016; Poteko et al., 2019; Qu et al., 2021; Sanchis et al., 2019).

Although the wind speed in the present study varied only in a narrow range between around 0 and  $0.5 \text{ m s}^{-1}$ , a significant effect on the  $\text{NH}_3$  emissions was apparent, in particular at high CP level in the diet (see Fig. 4). This observation is in line with several studies in naturally ventilated cubicle housings for dairy cows, which observed aside from temperature an effect of wind speed on  $\text{NH}_3$  release (e.g. Saha et al., 2014; Schrade et al., 2012; Wu et al., 2012). In contrast, in several meta-analyses the effect of wind speed on  $\text{NH}_3$  emissions was masked by more prominent driving parameters, e.g. temperature (Bougouin et al., 2016; Qu et al., 2021; Sanchis et al., 2019).

The CP content of the diet affects the urinary N excretion and thus also the level of  $\text{NH}_3$  emissions (e.g. Bougouin et al., 2016; De Boer et al., 2002; Devant et al., 2022; Edouard et al., 2019; Müller et al., 2021; Powell et al., 2008, 2011; Smits et al., 1995; Todd et al., 2006; Van Duinkerken et al., 2005, 2011). The milk urea content was identified in several studies as a relevant influencing variable on  $\text{NH}_3$  emissions (e.g. Powell et al., 2008; Schrade et al., 2012; Van Duinkerken et al., 2005, 2011). In some studies, the milk urea content was even called an indicator of  $\text{NH}_3$  emissions (Powell et al., 2011; Van Duinkerken et al., 2005, 2011; Wasem and Probst, 2022) because it reflected the N supply and excretion via urine. Thus, this could be used to estimate the baseline level of  $\text{NH}_3$  emissions. In our measurements, the milk urea content at low CP level was  $151 \text{ mg L}^{-1}$  and the  $\text{NH}_3$  emission on average  $17.3 \text{ g LU}^{-1} \text{ d}^{-1}$ . The mean  $\text{NH}_3$  emissions at high CP level were about 84 % higher with  $31.9 \text{ g LU}^{-1} \text{ d}^{-1}$  with a milk urea content of  $293 \text{ mg L}^{-1}$ . Thus, an increase in the milk urea content by  $10 \text{ mg L}^{-1}$  resulted in an increase in  $\text{NH}_3$  emissions by about 6 %. This is clearly higher than measurements in a Dutch dairy housing with cubicles and slatted floors in combination with restricted grazing, where  $\text{NH}_3$  emissions increased by 2.5 % and 3.5 % when the urea content of the milk was increased by  $10 \text{ mg L}^{-1}$  at levels between 200 and  $300 \text{ mg urea per litre of milk}$ , respectively (Van Duinkerken et al., 2011). At approximately the same milk urea range ( $210\text{--}300 \text{ mg L}^{-1}$ ), Powell et al. (2011) calculated  $\text{NH}_3$  emission reductions of 5 % to 7 % per  $10 \text{ mg L}^{-1}$  decrease based on five studies.

#### 4.4. Greenhouse gas emissions

The mean  $\text{CH}_4$  emissions of the two feeding variants barely differed numerically with  $316 \text{ g LU}^{-1} \text{ d}^{-1}$  (low CP) and  $310 \text{ g LU}^{-1} \text{ d}^{-1}$  (high CP). These values lie within the wide range of  $\text{CH}_4$  emissions from dairy housings with cubicles and solid floors varying from 168 to  $855 \text{ g LU}^{-1} \text{ d}^{-1}$ , which were mainly daily means, presented in a meta-analysis including 13 studies (Poteko et al., 2019). In a recent study, for the same experimental dairy housing, higher  $\text{CH}_4$  emission values ( $358 \text{ g LU}^{-1} \text{ d}^{-1}$ ) were measured over three seasons, but with a different herd composition and diet (Hempel et al., 2022). In contrast, the comparative averaged value over three seasons of a dairy housing with cubicles and solid floors in Germany was somewhat lower with  $274 \text{ g LU}^{-1} \text{ d}^{-1}$  (Hempel et al., 2022). Although  $\text{CH}_4$  emissions for both diets in our study were similar with respect to LU, they differed substantially relative to DMI and ECM. Differences between diets per DMI and ECM were possibly due to the lower DMI combined with the significantly (around 20 %) higher crude fibre content (see Table 1) in the diet with low CP content. Several studies and reviews have shown that a higher fibre content in the diet leads to higher enteric  $\text{CH}_4$  emissions (e.g. Aguerre et al., 2011; Benchaar et al., 2001; Knapp et al., 2014; Monteny et al., 2006; Niu et al., 2021). In respiration chamber experiments with dairy cows fed diets with different CP content

(138 vs.  $159 \text{ g CP per kilogram dry matter}$ ) but much smaller relative difference in the crude fibre content ( $164 \text{ vs. } 177 \text{ g crude fibre per kilogram dry matter}$ ), no significant effects on  $\text{CH}_4$  emissions were observed (Müller et al., 2021). Differences in the CP content (12 % vs. 18 % CP) might not always be accompanied by varying levels of NDF and ADF, and thus  $\text{CH}_4$  emissions, as reported by Edouard et al. (2019).

In contrast to  $\text{NH}_3$  and  $\text{CH}_4$  emissions, there have been rare measurements of  $\text{N}_2\text{O}$  emissions from dairy housings with cubicles, probably owing to limitations in online measurement techniques (Brewer et al., 2019). Mean  $\text{N}_2\text{O}$  emission values quantified in our study, with  $0.25 \text{ g LU}^{-1} \text{ d}^{-1}$  at low CP level and  $0.31 \text{ g LU}^{-1} \text{ d}^{-1}$  at high CP level, are at the total lower end of observations. Similar low but more variable  $\text{N}_2\text{O}$  emissions from  $0.3$  to  $2.5 \text{ g LU}^{-1} \text{ d}^{-1}$  were determined by Joo et al. (2015) in two buildings of a dairy facility predominantly equipped with cubicles. More than two orders of magnitude higher emissions than in our study, for both summer ( $42.2 \text{ g LU}^{-1} \text{ d}^{-1}$ ) and winter ( $47.2 \text{ g LU}^{-1} \text{ d}^{-1}$ ), were quantified by Samer et al. (2011). Most studies on  $\text{N}_2\text{O}$  emissions from cubicle housings for dairy cattle with solid or slatted floors reported two to ten times higher emission values compared with our measurements (Cortus et al., 2015; Huang and Guo, 2018; Jungbluth et al., 2001; Sneath et al., 1997). In this range also falls the  $\text{N}_2\text{O}$  emission value of  $2.47 \text{ g cow}^{-1} \text{ d}^{-1}$  derived for the Swiss N-flow model for slurry-based loose housing for dairy cattle by Kupper (2017). The  $\text{N}_2\text{O}$  emission factor for dairy cattle in loose housings in the Netherlands was considerably lower with  $0.88 \text{ g cow}^{-1} \text{ d}^{-1}$  (Mosquera and Hol, 2012).

Several  $\text{N}_2\text{O}$  emission measurements in the literature were done in feedlots or housing systems with non-structured lying areas. For instance, the  $\text{N}_2\text{O}$  emissions from feedlots with  $36.7 \text{ g cow}^{-1} \text{ d}^{-1}$  (Zhu et al., 2014) and  $10 \text{ g cow}^{-1} \text{ d}^{-1}$  (Leytem et al., 2011), from a corral system with  $4.1 \text{ g cow}^{-1} \text{ d}^{-1}$  (Owen and Silver, 2015) and from bedded packed dairy housings with  $1.6\text{--}17.0 \text{ g cow}^{-1} \text{ d}^{-1}$  (Van Dooren et al., 2016, 2019) are several times higher than our converted means with  $0.36 \text{ g cow}^{-1} \text{ d}^{-1}$  at low CP level and  $0.44 \text{ g cow}^{-1} \text{ d}^{-1}$  at high CP level. In these systems with (bare) soil or bedded areas in which aerobic and anaerobic conditions alternate, optimal conditions for nitrification and simultaneous denitrification prevail, which can lead to increased  $\text{N}_2\text{O}$  emissions (Almeida et al., 2022; Waldrip et al., 2016, 2020).

The effect of N excretion on  $\text{N}_2\text{O}$  emissions for dairy housing was discussed by Dijkstra et al. (2013) in a literature review but not experimentally tested up to now. In our study, average  $\text{N}_2\text{O}$  emissions per LU were reduced by almost 20 % in the low CP treatment compared with the high CP treatment. Although the difference is not as large as for  $\text{NH}_3$  emissions,  $\text{N}_2\text{O}$  emissions clearly reflect the CP level in the diet. Thus, a diet reduced in CP, which results in lower  $\text{N}_2\text{O}$  emissions, can also contribute to the reduction of GHG emissions, in line with observations for other ecosystems (Gruber et al., 2022). A positive correlation of temperatures and  $\text{N}_2\text{O}$  emissions was observed for a corral (Owen and Silver, 2015), a feedlot (Leytem et al., 2011), a cubicle housing (Cortus et al., 2015) and a housing with cubicles combined with pens (Huang and Guo, 2018).

$\text{CO}_2$  emissions with mean values of  $8678 \text{ g LU}^{-1} \text{ d}^{-1}$  at low CP level and  $9302 \text{ g LU}^{-1} \text{ d}^{-1}$  at high CP level in our study were somewhat lower than values averaging  $10,022 \text{ g LU}^{-1} \text{ d}^{-1}$ , which were measured in a naturally ventilated Canadian dairy housing with cubicles and pens (Huang and Guo, 2018), and higher than the range of  $5500$  to  $7500 \text{ g LU}^{-1} \text{ d}^{-1}$  measured in a mechanically ventilated cubicle dairy housing in Germany (Jungbluth et al., 2001). All the mentioned studies are within the range of  $5300$  to  $10,700 \text{ g LU}^{-1} \text{ d}^{-1}$  of a naturally ventilated freestall dairy housing in the USA (Joo et al., 2015). In respiration chamber experiments with diets containing different levels of CP ( $138 \text{ vs. } 159 \text{ g CP per kilogram dry matter}$ ), no significant differences in  $\text{CO}_2$  emissions were detected (Müller et al., 2021). The higher  $\text{CO}_2$  emissions in the treatment with a high CP level in our study may be due to the higher DMI and consequently a higher metabolic rate compared with the treatment with a low CP level. When  $\text{CO}_2$  emissions were expressed per DMI or per ECM, those at low CP level were slightly higher than those at high CP level (see Fig. 2).

## 5. Conclusions

Measurements on a practical scale using diets typical for Switzerland demonstrated that differences in CP content (17 % vs. 12 %) have a clear effect on both NH<sub>3</sub> and N<sub>2</sub>O emissions. On average, NH<sub>3</sub> emissions per LU were reduced by almost 50 % for the diet with low CP content compared with the diet with high CP content. For N<sub>2</sub>O emissions per LU, the mean reduction was around 20 %. Both NH<sub>3</sub> and N<sub>2</sub>O emissions were also significantly reduced for the low CP diet related to DMI and ECM. The results are supported by strong relations between the CP content of the diet, the N excretion via urine and the milk urea content. Thus, with targeted control and adjustment of feeding, NH<sub>3</sub> and N<sub>2</sub>O emissions can be markedly reduced. For this purpose, the milk urea content can be used as an indicator in the case of lactating cows. With regard to CH<sub>4</sub> emissions, the crude fibre content of the diet and the DMI also have to be taken into account. Optimising the N content at the feeding stage takes place at the beginning of the N chain and offers great potential for implementation because no additives or constructional changes of buildings are necessary.

## CRedit authorship contribution statement

**Sabine Schrade:** Conceptualization, Methodology, Investigation, Data curation, Visualization, Writing – original draft, Resources, Project administration, Funding acquisition. **Kerstin Zeyer:** Investigation, Methodology, Data curation, Writing – review & editing. **Joachim Mohn:** Conceptualization, Methodology, Writing – review & editing, Resources, Funding acquisition. **Michael Zähler:** Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – review & editing, Resources.

## Data availability

Data will be made available on request.

## Declaration of competing interest

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