An energy-protein feed additive containing different sources of fat improves feed intake and milk performance of dairy cows in mid-lactation

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SUPPLEMENTARY FILE

Detailed methodology of research

Material & experimental design

The study was carried out according to the guidelines of the Local Ethics Committee for Experiments on Animals in Kraków. The trial was conducted at a free-stall barn dairy farm (OHZ Osięciny Sp. z o.o., 400 cows, with average milk yield of about 11,000 kg per lactation) located in north-western Poland. DMI was measured using Calan gates (American Calan, New Hampshire, USA). The experimental TMR was mixed in a Super Data Ranger feeding wagon (SDR, American Calan, New Hampshire, USA). The amount of refusals was measured daily. The cows were milked twice daily in a fishbone milking parlour. The entire trial lasted 9 weeks (63 days) and consisted of a 3-week pre-treatment period and a 6-week period of data collection.

Forty multiparous Holstein-Friesian mid-lactation dairy cows were divided randomly into 4 groups of 10 cows each with average milk yield (42·2 kg/d), average DIM (67) and average parity (3·0). The TMR diet was formulated using CPM-Dairy software (CAHP, University of Pennsylvania, USA) based on NRC (2001) recommendations for a 670 kg cow producing 50 kg/d of milk containing 38 g/kg of milk fat and 32 g/kg crude protein. The treatments were as follows: TMR without the experimental energy-protein feed additive (EFA-0) or with 1 kg (EFA-1), 2 kg (EFA-2) or 3 kg (EFA-3) of the additive. The share of EFA in the dry matter of the experimental diets in the four treatments was 0%, 3·2%, 6·4%, and 9·5%, respectively. Experimental TMR were balanced for protein and energy content

using high-moisture maize silage and soybean meal (Table 1).

EFA contained whole linseeds of the yellow variety (30·30%), linseed and fish oil Ca-salts (1:1) manufactured according to our own formula (23·74%), high-fat heat-treated rapeseed cake (28·04%), wheat bran (15·82%), Blattin Lacto-Fatt (calcium salt of palm oil fatty acid; 1·68%) and mineral & vitamin mix (0·42%; in 1 kg: calcium 192 g, sodium 0·3 g,

vitamin E 37,500 mg, β-carotene 40,000 mg, iodine as calcium iodate 1500 mg, selenium as sodium selenite 750 mg). Ca-salts were considered a protected fat and a good source of PUFA available in the small intestine. The content of minerals and vitamins in the EFA (Table 2) was not taken into account when balancing minerals and vitamins in the diet. The control diet covered NRC (2001) requirements, while EFA-1, EFA-2 and EFA-3 diets exceeded NRC requirements for vitamins A and E, iodine and selenium.

Sampling and chemical analysis of feed, TMR and refusals

Samples of individual feeds, TMRs and refusals of each cow were collected daily, stored at +2°C and then pooled weekly and kept frozen (−18°C) for further analysis. DMI was calculated from DM content determined by drying TMRs and refusals in a forced-air oven at 50°C for 48 hours. In the dried and ground (1 mm sieve) samples of feed, TMRs and refusals, the content of dry matter, ash, crude protein, ether extract and acid detergent fibre (ADF) were determined according to AOAC (2004) (procedure numbers 934.01, 942.05, 954.01, 920.39 and 973.18, respectively). Neutral detergent fibre (aNDF) was determined according to van Soest et al. (1991) using an Ankom^{220} Fiber Analyzer (ANKOM Technology, NY, USA) with heat-stable amylase and expressed inclusive of residual ash. Starch content was determined by an enzymatic method (Faisant *et al.* 1995). The FA profile was determined using a Varian 450-GC gas chromatograph (Varian BV, Middelburg, The Netherlands) with an FID detector, equipped with a CP-SIL 88 column (FAME, length 100 m, diameter 0·25 mm).

Milk analysis

Milk was collected individually from each animal once a week during the collection period. Milk samples from morning and evening milking were pooled proportionally and

stored with Microtabs II (Bentley). For determination of FA profile, cholesterol, phospholipids and fat-soluble vitamins, samples from each animal were collected only from morning milking, cooled (4°C), and then immediately frozen.

Milk protein, fat, lactose and urea were determined with a MilkoScan FT2 (Foss Analytical). Milk FA profile was determined by gas chromatography (GC) using a Varian 450-GC apparatus with an FID detector and a CP-SIL 88 column (FAME, length 100 m, diameter 0·25 mm). The sum of phospholipids in the milk fat and their individual classes were determined by high-performance liquid chromatography (HPLC) with a Dionex UltiMate 3000 apparatus (Thermo Fisher Scientific, Waltham, MA USA) equipped with a Corona CAD detector and a Thermo Betasil DIOL 5 µm (150 × 4·6 mm) column according to Kiełbowicz *et al.* (2013). Lipids were extracted from the milk by the Folch *et al.* (1957) method. Cholesterol content was determined by capillary GC (Trace GC Ultra (Thermo Electron Corporation) with a HP-5 column), using the method of direct saponification for the sample preparation according to Fletouris *et al.* (1998). The HPLC method (Agilent 1100 apparatus (Agilent Technologies, Santa Clara, CA, USA) equipped with a Zorbax Eclipse XDB-C8 column with a diameter of 4·5 mm x 150 mm) was used for determination of fat soluble vitamins (A, D, E and K) and β-carotene. The method involved saponification at room temperature and subsequent extraction of vitamins with n-hexane. The vitamins were resolved with a C18 reversed-phase column and detected by UV spectrophotometry (Albalá-Hurtado *et al.* 1997).

Statistical analysis

Data were analysed as a completely randomized block design using the MIXED procedure of SAS (SAS, 2002). The statistical model for repeated variables included the effect of time (week) and the interaction between the effects of time and treatment as fixed

effects (Littell *et al.* 1998). The optimal covariance structure (autoregressive order one, unstructured or compound symmetry) was chosen based on Akaike's criterion. For all analysed parameters, initial yield or composition of milk was used as a covariate. The significance level was set at $P \leq 0{\cdot}05$. Tendencies were discussed at $0{\cdot}05 < P < 0{\cdot}10$ unless otherwise stated. Data are presented as least squares means and corresponding SEM.

Table 1. - follow-up

[1]Blend Multi (%): soybean meal 43·57, urea 5·88, Farm Pack 2·94, Vitosa Biot Plus 10·59, Acid Buff 4·71, limestone 16·7, sodium chloride 5·88, magnesium sulphate 5·88, magnesium oxide 3·56, Rumex 0·29 (g in DM: CP 435, EE 14, NDF 86)

Table 2. Chemical composition (g/kg DM), net energy lactation content (NEL, Mcal/kg DM) and fatty acid profile (g/100g FA) of experimental feed additive (EFA)

Item	Content
Dry matter	936
Crude ash	82
Crude protein	198
Crude fat (ether extract)	349
aNDF	300
ADF	190
Starch	24
Selenium, mg	3·80
Iodine, mg	6·81
B-carotene, mg	191·6
Vitamin E, mg	202·4
NEL	2·85
C12:0	0·03
C14:0	0·85
C14:1	0·04
C15:0	0·07
C16:0	7·96
C16:1	1·41
C18:0	3·10
C18:1	37·40
C18:2	17·33
C18:3	28·23
C20:0	0·19
C20:1	1·42
C20:2	0·13
C20:3	0·07
C20:4	0·06
C20:5	0·16
C22:1	0·78
C22:5 (EPA)	0·05
C22:6 (DHA)	0·10
CLA, 9c 11t	0·00
Others	0·62

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