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# Changes in Fatty Acid Levels during In Vitro Ruminal Fluid Incubation with Different Proportions of Maize Distillers Dried Grains (DDGS)

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**Abstract:** This study aimed to analyse changes in the profile of long-chain fatty acids in the ruminal fluid of cows during in vitro fermentation, using different proportions of maize DDGS (distillers dried grains with solubles) as a substrate. The serum bottles were filled with 1 g of concentrate feed (C), which consisted of cereal middlings, postextraction rapeseed meal, and soybean meal. Substrates I, II, and III contained the same ingredients as substrate C, but also included DDGS at increasing proportions, while substrate IV contained only DDGS. Ruminal fluid with a buffer was then added to the bottles and incubated for 4, 8, and 24 h. After incubation, the fatty acid profile was analysed using a gas chromatograph. The use of DDGS as a substrate resulted in a decrease in SFA, and an increase in the proportion of UFA, including oleic acid (C18:1n9c) and linoleic acid (C18:2n6c). The fermentation profile with 15% and 20% DDGS in TMR proved to be the most beneficial. These findings suggest that the byproduct of bioethanol production could potentially improve the fatty acid profile in the ruminal fluid, resulting in higher-quality animal products.

**Keywords:** cows; maize DDGS; rumen; in vitro microbial processes; fatty acid profile



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## 1. Introduction

Bioethanol production results in an overproduction of distillers dried grains with biological substances dissolved in them (DDGS) [1,2]. Using byproducts from the agri-food industry may be an alternative to the traditional animal feed system. It is also a way to naturally utilize these products and their valuable nutrients [3]. The global need to find new energy sources has led to increased biofuel production in recent years, emerging as an alternative to the extraction of fossil raw materials [4,5]. However, it is associated with significant quantities of byproducts that must be disposed of, with distillers grains being one of these byproducts, which can be used as livestock feed after appropriate technological processing and meeting microbiological and toxicological standards [6–9].

Ongoing research indicates that maize distillers dried grains can be used in feeding dairy cows as an additive or substitute for other concentrate feeds, without adverse effects on milk performance traits [10,11]. Additionally, substituting starch and protein from maize, other cereals, or soy with cheaper feeds, such as DDGS, reduces the cost of feeding cows [12]. Compared to maize grain, maize DDGS has a much lower content of

starch, which is fermented during ethanol production. Nevertheless, most other grain constituents, including protein and long-chain fatty acids, remain unchanged during this process and are a valuable source of nutrients as part of DDGS. In this situation, fatty acids are one of the main sources of energy in maize DDGS [9].

In the rumen, lipolysis results in the hydrogenation of C18:2n-6 and C18:3n-3 acids to both cis- and trans-C18:1 intermediates, followed by the formation of stearic acid [13]. The process of biohydrogenation significantly impacts the quality of animal products. Foods high in long-chain unsaturated acids are desirable in the human diet [14]. DDGS has a high crude fat content and is also a source of polyunsaturated fatty acids, which provide some protection against biohydrogenation processes in the rumen and may improve the quality of animal products [13].

This in vitro study is a part of a project aiming to introduce maize DDGS into the diet of cows during their dry period. This study aimed to analyse the fatty acid profiles in the ruminal fluid of cows during in vitro incubation, using varying proportions of maize distillers dried grains in a fermentable substrate for rumen microbiota.

## 2. Materials and Methods

### *2.1. Animals*

The material for the study consisted of ruminal fluid collected with a rumen probe from eight Polish Holstein-Friesian cows in the drying-out period. The animals were kept in a free-stall system and fed TMR, with a ration composed of 52.21% maize silage, 9.1% haylage, 2.0% straw, 7.25% ensiled beet pulp, 9.36% cereal middlings (50% wheat, 50% barley), 9.52% postextraction rapeseed middlings, 7.62% soybean meal, 0.97% mineral-vitamin premix, 0.97% sodium bicarbonate, 0.57% chalk, 0.42% protected fat, and 0.01% dried yeast (Table 1). The feed ratio was prepared under INRA standards [15].

**Table 1.** Diet nutritional value of cows (g/kg DM).

| | |
|---|---|
| UFL kg DM | 0.79 |
| PDIN g/kg DM | 82 |
| PDIE g/kg DM | 79 |
| FV kg/DM | 0.75 |
| DCAD mEq/kg DM | 189 |

UFL—unit for lactation, PDIN—the sum of microbial protein that could be synthesised in the rumen from available N, and the dietary protein undegraded in the rumen but truly digestible in the small intestine, PDIE—the sum of microbial protein that could be synthesised in the rumen from available energy, and the dietary protein undegraded but truly digestible in the small intestine, FV—fill value, DCAD—dietary cation–anion difference.

### *2.2. In Vitro Incubation*

Since DDGS is recommended as a substitute for concentrate rather than roughage, the concentrate feed components of the cows' rations were used to create the in vitro substrates for ruminal fluid microbiota. The components of the substrates were used in the same proportions as concentrate components in TMR for cows, in an in vivo experiment, which was a part of the same research project as our study. In the control group (C), the substrate contained only concentrated feed ingredients in the same proportions as the basal diet TMR (cereal middlings, postextraction rapeseed meal, and soybean meal). Substrates I, II, and III contained the same components, but with the addition of DDGS in amounts equivalent to 10%, 15%, and 20% of the dry matter of the total TMR ratio, while substrate IV contained only DDGS (Table 2).

**Table 2.** Composition, chemical analysis, and nutritional value of substrates used in the in vitro experiment.

| Item | C | DDGS | | | |
|---|---|---|---|---|---|
| | | I | II | III | IV |
| Dry matter DM (% of diet) | 88 | 89.5 | 90.3 | 91 | 91.8 |
| Crude protein (% DM) | 40.41 | 37.83 | 36.49 | 34.63 | 29.40 |
| Ether extract (crude fat) (% DM) | 1.44 | 5.91 | 8.66 | 10.76 | 13.29 |
| Crude fibre (% DM) | 10.03 | 11.31 | 10.88 | 11.11 | 10.35 |
| NDF (% DM) | 30.31 | 36.22 | 38.53 | 41.06 | 43.54 |
| ADF (% DM) | 13.66 | 15.38 | 14.80 | 15.09 | 14.07 |
| N-free extract (% DM) | 56.35 | 50.94 | 48.62 | 47.16 | 49.84 |
| Crude ash (% DM) | 5.39 | 5.88 | 6.07 | 6.20 | 5.95 |
| Energy UFL/kg DM | 10.05 | 10.03 | 10.03 | 10.02 | 10.02 |
| feed ingredients (%) | | | | | |
| Cereal meal (50% barley, 50% wheat) | 35.7 | 16.8 | 7.4 | 0 | 0 |
| Extracted rapeseed meal | 35.7 | 32.8 | 19.1 | 14.1 | 0 |
| Extracted soybean meal | 28.6 | 14.6 | 14.7 | 8.5 | 0 |
| DDGS | 0 | 35.8 | 58.8 | 77.4 | 100 |
| g/100 g of total fat concentration | | | | | |
| C14:0 | 0.00 | 0.00 | 0.04 | 0.00 | 0.03 |
| C15:0 | 0.00 | 0.13 | 0.00 | 0.00 | 0.00 |
| C16:0 | 8.13 | 10.67 | 8.32 | 11.08 | 10.58 |
| C18:0 | 1.53 | 2.00 | 1.71 | 1.94 | 1.92 |
| C20:0 | 0.00 | 0.40 | 0.44 | 0.43 | 0.40 |
| C14:1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.04 |
| C18:1n9c | 16.73 | 25.73 | 29.96 | 27.81 | 28.54 |
| C18:1n7t | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| C18:2n6c | 27.71 | 48.88 | 53.79 | 51.95 | 54.31 |
| C18:3n3 | 4.19 | 2.45 | 1.68 | 1.59 | 1.16 |

NDF—neutral detergent fibre, ADF—acid detergent fibre.

Eight cows were ruminal fluid donors (n = 8). The ruminal fluid from each cow was used as inoculum for five in vitro samples: C, I, II, III, and IV (representing 8 replicates per each substrate), and then three bottles per each substrate and inoculum were used for incubation, one for each incubation time (4, 8, 24 h). To prepare the samples for the in vitro incubation, the collected ruminal fluid was filtered through gauze, and 30 mL of the fluid was transferred into 125 mL serum bottles (Sigma-Aldrich) and then diluted three times with buffer [16]. Five samples were prepared from the diluted ruminal fluid taken from each animal, to which 1 g of one of the previously prepared substrates was added: C, I, II, III, or IV. To achieve anaerobic conditions, the bottles were saturated with carbon dioxide from a pressurised bottle and then closed tightly with a capping machine. Each sample was prepared in triplicate and incubated in a shaker with a water bath at 39 °C for four, eight, and twenty-four hours.

### *2.3. Fatty Acids Analysis*

Fat was extracted from the substrate and liquid samples after fermentation for 4, 8, and 24 h, according to the Folch method [17]. Fatty acid methyl esters were obtained according to the method of Christopherson and Glass [18], using a solution of 2M KOH in methanol. The fatty acid profile of the samples obtained was determined using an Agilent Technologies 7890A gas chromatograph (Santa Clara, CA, USA) with the FID detector. The determinations were carried out under the following conditions: HP-88 capillary column (Agilent Technologies), 100 m long, 0.25 mm diameter, and 0.20 µm film thickness, with an initial oven temperature of 50 °C and a temperature rise of 3 °C/min up to 220 °C; detector and dispenser temperatures of −270 °C and 270 °C respectively, helium as the carrier gas. In addition, hydrogen, synthetic air, and nitrogen flowed through the detector. Identification of the obtained fatty acid peaks was performed by comparison with the retention

times of Sigma-Aldrich fatty acid methyl ester standards using ChemStation software (Agilent Technologies).

### *2.4. Chemical Analyses*

The primary nutrients were determined in representative samples: Dry matter (DM; method 934.01 of Association of Official Agricultural Chemists—AOAC) [19]. Crude protein (CP; Kjeldahl method, method 984.13 of AOAC 2005), using a Kjeltec 2300 Foss Tecator apparatus (Häganäs, Sweden) and by multiplying the nitrogen content by 6.25 (CP; Kjeldahl method, method 984.13 of AOAC) [19]. Ether extract (EE; method 920.39 of AOAC 2005), using a Fibertec Tecator (Häganäs, Sweden) apparatus (CF; method 978.10 of AOAC) [19]. Crude ash was determined by method 942.05 of AOAC [19]. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) fractions were determined using a Fibertec Tecator (Häganäs, Sweden) apparatus (NDF; method of Holst,1973 [20]) and acid detergent fibre (ADF; method 973.18 of AOAC [19]). The net energy of lactation (NEL) in a unit for lactation (UFL) was estimated according to the INRA feeding system [15]. The nonfibre nostructural carbohydrate (NFSC) content was calculated according to the National Research Council [21], as follows: 100 − (Ash + CP + EE + NDF), and nitrogen-free extractives (NFE) were calculated as 100 − (Ash + CP + EE + CF), with ash, CP, EE, CF, and NDF contents expressed as % of DM. DCAD—dietary cation–anion difference was calculated using the formula (Na + K + 0.38 Ca + 0.30 Mg) − (Cl + 0.6 S + 0.5 P) [22]. FV, PDIE, and PDIN utilization was calculated according to the method of INRA [15].

### *2.5. Statistics*

The study results were statistically processed by a split s-plot design analysis [23] using R software [24], according to the following model:

$$y\_ijk = \mu + \llbracket \tau\_i + \beta \rrbracket\_j + \gamma\_k + \llbracket (\beta\gamma) \rrbracket\_jk + \varepsilon\_ijk$$

where y_ijk is the dependent variable under examination, μ is the overall mean, τ_i is the block effect represented by cow i, β_j is the main-plot factor represented by the substrate type j, and γ_k is the subplot factor represented by fermentation time k. ⟦(βγ)⟧_jk is the interaction effect between the substrate type j and fermentation time k. ε_ijk is the residual term. It is assumed that main- and subplot effects are fixed factors and the block effect is random.

The effects of fermentation time (4, 8, 24 h) and substrate type (C, I, II, III, IV) were analysed. The significance at $p < 0.05$ of differences between groups was determined using Duncan's multiple comparison test. The Pearson correlation coefficients between fatty acid levels and DDGS inclusion were also calculated.

## 3. Results

The total content of the examined fatty acids in the substrates (g/100 g fat) was higher than in the control group, but their proportion was similar between the experimental groups (Table 2). As a result, the variable DDGS content in the samples studied—ranging from 10% to 100%—had no effect on the fatty acid content of the substrates per 100 g of fat. Only trace amounts of C14:0, C15:0, C14:1, or C18:1n7t acids were found. The levels of C18:1n9c and C18:2n6c acids increased with the growing proportion of DDGS in the samples. In group IV, it was more than 70% and almost twice as high as in the control group, respectively. The increasing proportion of DDGS in the samples resulted in a significant systemic decrease in C18:3n3 acid content. The higher total content of polyunsaturated fatty acids in the substrate samples as the proportion of DDGS increased is mainly due to a systematically increasing fat content in DDGS compared to the control group. The fat content of the individual samples ranged from 1.44 (% dry matter) in the control group to 13.29 in group IV (5.91 in I, 8.66 in II, and 10.76 in III).

The effect of adding maize DDGS as a part of the substrate on the fatty acid profile in the rumen contents of cows during in vitro fermentation is shown in Table 3.

**Table 3.** Effect of time and the proportion of DDGS included in the substrate on saturated fatty acid levels in ruminal fluid incubated in vitro (n = 8).

| Fatty Acid [1] | 4 h | | | | | 8 h | | | | | 24 h | | | | | SEM | | Significanceof Split-Plot Design | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | DDGS | | | | C | DDGS | | | | C | DDGS | | | | | | | | |
| | | I | II | III | IV | | I | II | III | IV | | I | II | III | IV | Main Plot | Sub Plot | s | t | s x t |
| C4:0 | 1.00 | 0.46 | 0.51 | 0.44 | 0.39 | 1.18 | 0.94 | 0.89 | 0.52 | 0.42 | 1.28 | 0.65 | 0.72 | 0.58 | 0.44 | 0.126 | 0.091 | * | NS | NS |
| C6:0 | 0.44 | 0.42 | 0.29 | 0.00 | 0.33 | 0.68 | 0.87 $^{a}$ | 0.59 | 0.00 $^{b}$ | 0.09 | 1.12 | 0.72 | 1.33 | 0.74 | 0.84 | 0.043 | 0.052 | * | NS | NS |
| C11:0 | 0.90 | 0.96 | 0.65 | 0.17 | 0.17 | 1.35 | 0.91 | 2.27 $^{a}$ | 0.18 $^{b}$ | 0.77 | 1.15 | 0.67 | 1.17 | 0.38 | 0.59 | 0.133 | 0.120 | * | NS | NS |
| C14:0 | 1.61 | 1.70 | 2.00 | 0.63 | 0.50 | 2.29 | 1.65 | 1.13 | 0.60 | 0.59 | 4.49 $^{a}$ | 3.72 $^{ab}$ | 1.26 $^{b}$ | 1.19 $^{b}$ | 2.20 $^{ab}$ | 0.253 | 0.262 | * | * | NS |
| C16:0 | 18.45 | 19.81 | 21.41 | 17.55 | 18.60 | 21.24 | 17.96 | 18.16 | 17.72 | 17.49 | 20.71 $^{bc}$ | 24.77 $^{a}$ | 17.35 $^{c}$ | 18.24 $^{c}$ | 19.71 $^{c}$ | 0.950 | 0.654 | NS | NS | * |
| C18:0 | 8.81 | 7.83 | 10.10 | 9.03 | 5.27 | 11.23 | 8.20 | 8.83 | 7.61 | 6.21 | 10.03 | 11.56 | 10.21 | 10.71 | 11.77 | 1.074 | 0.528 | NS | * | NS |
| C20:0 | 0.71 | 1.02 | 0.73 | 0.59 | 0.62 | 0.64 | 0.70 | 0.56 | 0.64 | 0.67 | 0.54 | 0.34 | 0.57 | 0.70 | 0.59 | 0.071 | 0.070 | NS | NS | NS |
| Σ SFA | 30.99 | 32.91 | 36.15 | 27.32 | 25.32 | 38.25 | 30.79 | 30.64 | 27.22 | 25.76 | 39.25 $^{ab}$ | 43.67 $^{a}$ | 30.64 $^{b}$ | 32.02 $^{b}$ | 36.69 $^{ab}$ | 2.025 | 1.346 | NS | * | NS |
| C14:1 | 0.70 $^{ab}$ | 1.33 $^{a}$ | 0.47 $^{b}$ | 0.65 $^{ab}$ | 0.43 $^{b}$ | 0.85 | 0.69 | 0.58 | 0.55 | 0.82 | 1.27 | 0.84 | 0.86 | 0.83 | 0.86 | 0.000 | 0.066 | NS | NS | * |
| C16:1 | 0.94 $^{b}$ | 1.75 $^{a}$ | 0.93 $^{ab}$ | 0.32 $^{ab}$ | 0.16 $^{b}$ | 0.69 | 1.13 | 0.92 | 0.27 | 0.63 | 1.24 | 1.03 | 0.68 | 0.93 | 0.72 | 0.099 | 0.146 | * | NS | NS |
| C17:1 | 0.68 | 2.50 $^{a}$ | 0.00 $^{b}$ | 1.46 | 0.44 | 0.83 | 2.11 | 1.25 | 0.57 | 1.19 | 1.99 | 0.74 | 1.08 | 0.80 | 0.68 | 0.147 | 0.206 | NS | NS | NS |
| C18:1n9c | 17.82 | 19.10 | 18.78 | 20.28 | 24.34 | 15.08 | 15.18 | 16.58 | 20.65 | 21.27 | 11.28 $^{b}$ | 17.10 $^{b}$ | 15.93 $^{ab}$ | 18.84 $^{ab}$ | 20.58 $^{a}$ | 1.295 | 0.864 | * | NS | NS |
| C18:1n7t | 5.69 | 2.03 | 2.25 | 1.66 | 0.67 | 2.27 | 1.24 | 0.80 | 2.10 | 1.60 | 2.11 | 1.71 | 5.20 | 8.45 | 5.27 | 0.587 | 0.637 | NS | * | NS |
| C18:2n6c | 20.58 $^{b}$ | 20.88 $^{b}$ | 27.23 $^{ab}$ | 30.47 $^{ab}$ | 41.35 $^{b}$ | 19.82 $^{b}$ | 22.01 $^{ab}$ | 30.39 $^{ab}$ | 36.37 $^{a}$ | 34.97 $^{a}$ | 8.76 $^{b}$ | 26.05 $^{b}$ | 17.12 $^{b}$ | 21.17 $^{a}$ | 18.56 | 1.620 | 1.562 | * | * | NS |
| C18:3n3 | 3.25 $^{a}$ | 1.53 $^{b}$ | 1.51 $^{b}$ | 1.48 $^{b}$ | 1.22 $^{b}$ | 3.32 $^{a}$ | 1.67 $^{b}$ | 1.82 $^{b}$ | 1.66 $^{b}$ | 1.28 $^{b}$ | 1.70 | 2.15 | 1.33 | 1.30 | 0.92 | 0.114 | 0.090 | * | * | NS |
| C20:1 | 1.26 | 3.41 | 0.65 | 0.97 | 1.79 | 0.74 | 1.17 | 0.76 | 2.16 | 0.79 | 18.12 | 1.15 | 2.35 | 2.37 | 3.25 | 0.324 | 0.269 | NS | NS | * |
| C20:4n6 | 0.24 | 2.98 | 13.51 | 1.86 | 1.08 | 2.26 | 2.27 | 6.83 | 0.00 | 12.83 | 0.00 | 5.27 | 6.86 | 4.86 | 2.39 | 1.381 | 0.627 | * | * | NS |
| EPA | 0.36 | 0.30 | 0.34 | 0.38 | 0.32 | 0.00 $^{b}$ | 0.37 $^{a}$ | 0.31 $^{a}$ | 0.37 $^{a}$ | 0.37 $^{a}$ | 0.00 $^{c}$ | 0.54 $^{a}$ | 0.37 $^{b}$ | 0.43 $^{b}$ | 0.27 $^{b}$ | 0.023 | 0.011 | * | NS | NS |
| Σ UFA | 50.65 $^{b}$ | 58.59 $^{ab}$ | 52.55 $^{ab}$ | 56.35 $^{ab}$ | 69.77 $^{a}$ | 46.73 | 47.92 | 53.52 | 63.07 | 64.13 | 44.10 | 43.99 | 50.36 | 54.88 | 53.60 | 2.715 | 2.152 | * | NS | NS |

[1] g/100 g of total fat concentration; SFA—saturated fatty acids; C, I, II, III, IV—see Table 2 for substrate compositions; SEM—standard error of the mean; t—effects of fermentation time; s—effects of substrate; s x t—the interaction effects between substrate and fermentation time; NS—not significant, * $p < 0.05$; mean values in rows marked with different lowercase letters (a, b, c) differ significantly at $p < 0.05$.

The effect of fermentation time on the proportions of tetradecanoic acid (C14:0), octadecanoic acid (C18:0), vaccenic acid (C18:1n7t), linoleic acid (C18:2n6c), linolenic acid (C18:3n3), and total saturated acids (SFA) in cow ruminal fluid was found to increase ($p < 0.05$) (Table 3). At eight hours of in vitro fermentation, the level of C6:0 acid in the ruminal fluid was higher ($p < 0.05$) in group I than in group III. Furthermore, at 8 h of fermentation, group II had a higher ($p < 0.05$) proportion of undecanoic acid (C11:0) than group III. At 24 h of fermentation, there was an increase ($p < 0.05$) in the level of C14:0 acid in the control group compared to groups II, III, and IV. The level of this acid was also higher ($p < 0.05$) in group I compared to groups II and III. The proportion of C16:0 acid at 24 h of fermentation was higher ($p < 0.05$) in group I than in groups II, III, and IV. Changes in the concentration of SFA in the ruminal fluid were recorded at 24 h of fermentation, with a higher ($p < 0.05$) proportion in group I compared to groups II and III. The longest time of fermentation significantly ($p < 0.05$) reduced the levels of unsaturated acids, such as vaccenic (C18:1n7t) and α-linolenic (C18:3n3), and the changes in the levels of linoleic acid (C18:2n6c), eicosapentaenoic acid (EPA), and total unsaturated fatty acids (UFA) were highly significant (Table 3). The substrates used in this study caused an increase ($p < 0.05$) in the total UFA concentration. Furthermore, the inclusion of DDGS caused an increase in the proportions of oleic acid (C18:1n9c), C18:2n6c, 20:4n6, and EPA, but a decrease in C18:3n3 proportions in the fermented ruminal fluid ($p < 0.05$). The substrates used in the in vitro incubation of cow ruminal fluid lowered the levels of unsaturated fatty acids, such as C4:0, C6:0, C11:0, and C14:0. The interactions between substrate inclusion and fermentation time were also observed for the acids C16:0, C14:1, and C20:1 ($p < 0.05$).

In vitro fermentation of ruminal fluid with different proportions of DDGS in the substrate affected the unsaturated fatty acid profile. At 4 h of fermentation, the balance of cis-9-tetradecenoic acid (C14:1) and cis-9-heptadecanoic acid (C17:1) in group I was higher ($p < 0.05$) than in group II. C14:1 and cis-9-hexadecane (C16:1) levels at 4 h of fermentation increased ($p < 0.05$) in group I as compared to group IV. At 4 and 8 h of fermentation, the proportion of C18:3n3 acid in group C was higher ($p < 0.05$) compared to groups where DDGS was used in the substrate. On the other hand, the level of EPA acid at 8 and 24 h of fermentation was higher ($p < 0.05$) in groups where DDGS was used, compared to group C. Additionally, at 24 h of fermentation in group II, the level of this acid was higher ($p < 0.05$) compared to group IV. However, the most remarkable changes were observed for C18:2n6c acid: at 4 and 8 h of fermentation, lower ($p < 0.05$) levels were observed in group C with respect to groups III and IV. In contrast, at 24 h of fermentation, the proportion of C18:2n6c acid was lower ($p < 0.05$) in group C with respect to group III. At 4 h of fermentation, there was an increased ($p < 0.05$) proportion of C18:2n6c acid in group IV with respect to groups I and II. At 24 h of fermentation in ruminal fluid fermented with DDGS, group IV had an increased ($p < 0.05$) level of C18:1n9c with respect to groups C and I. UFA levels at 4 h of in vitro fermentation were higher in group IV compared to group C.

In addition, we estimated the Pearson correlation coefficients between the levels of individual fatty acids and the proportion of DDGS (Table 4). The increasing proportion of DDGS was strongly correlated with increasing concentrations of EPA, C18:2n6c, and C18:1n9c, as well as total UFA concentration in ruminal fluid after 8 h of incubation. Significant negative correlations were observed between DDGS and total SFA concentration, as well as the individual saturated FAs C14:0 and C:18, over the same period, while a significant negative correlation was also observed for the unsaturated acid C20:4n6. Weaker correlations were found at 4 and 24 h of incubation.

**Table 4.** Pearson correlation coefficients between fatty acids and DDGS inclusion.

| Fatty Acid | 4 h | 8 h | 24 h |
|---|---|---|---|
| C4:0 | −0.253 | −0.217 | −0.101 |
| C6:0 | −0.098 | −0.015 | −0.073 |
| C11:0 | −0.127 | −0.276 | −0.203 |
| C14:0 | −0.308 | −0.637 * | −0.493 * |
| C16:0 | −0.006 | −0.428 | −0.386 * |
| C18:0 | −0.130 | −0.487 * | −0.003 |
| C20:0 | −0.005 | −0.090 | 0.297 |
| Σ SFA | −0.149 | −0.572 * | −0.413 * |
| C14:1 | −0.104 | −0.261 | −0.329 |
| C16:1 | −0.210 | −0.351 | −0.283 |
| C17:1 | −0.094 | −0.193 | −0.123 |
| C18:1n9c | 0.277 | 0.669 * | 0.288 |
| C18:1n7t | −0.234 | 0.071 | 0.359 * |
| C18:2n6c | 0.442 * | 0.766 * | 0.391 * |
| C18:3n3 | −0.649 * | −0.095 | −0.243 |
| C20:1 | −0.063 | 0.478 | 0.383 * |
| C20:4n6 | 0.054 | −0.699 * | −0.397 * |
| EPA | 0.222 | 0.830 * | 0.378 * |
| Σ UFA | 0.274 | 0.704* | 0.275 |

* Correlation coefficient significant at $p < 0.05$.

## 4. Discussion

The manipulation of ruminal lipid metabolism, aimed to improve the fatty acid profile in animal products, has often been the subject of scientific research [25]. As changing the diet composition and introducing feed additives in animal diets are connected with alterations of the microbial activity, often causing changes in the ruminal lipid profile, the effect of introducing new feed components on ruminal fatty acid profiles should always be investigated. In our study, we analysed changes in the fatty acid profile in ruminal fluid, which was influenced by introducing maize DDGS. A previous study indicated that the use of maize DDGS supplementation increased the fat content of the TMR [26].

Similarly, using 10% and 20% DDGS as an additive resulted in higher fat levels in the feed ratio. It also caused a higher proportion of unsaturated fatty acids in the ruminal fluid, while saturated acids were reduced [27]. In our research, we stated a similar relationship: ether extract content in substrate DM increased with an increase of DDGS. A high proportion of fat alters the fermentation profile in the rumen, which may influence animal products. A reduction in methane production and an increase in the milk yield of cows at 6% ether extract in DM of TMR are indicated by the results of Patra [28]. An increase in the proportion of polyunsaturated acids in the feed reduces methanogenesis in the rumen [28]. Our previous in vitro study indicated a reduction in methanogenesis as a result of increased DDGS in the fermentable substrate [29]. Due to its bonding with unsaturated acids, hydrogen is not used for methane production [30,31]. The fat in DDGS is mainly composed of unsaturated fatty acids, with C18:2 and C18:1n9c together accounting for about 50% and 25% of the total fatty acids, respectively [32].

In our study, the use of DDGS as a substrate increased the levels of unsaturated acids, such as C18:1n9c, C18:2n6c, EPA, and total UFA, in the incubated ruminal fluid. However, it decreased the level of C18:3n3. These results were mainly related to the level of unsaturated fatty acids in the fermentation substrates, as the addition of DDGS resulted in an increase in C18:1n9c and C18:2n6c acids, but a decrease in C18:3n3 acid in the substrate. The absence of 20-carbon fatty acids in the substrates, and their presence in rumen-incubated samples, indicates the origin of these acids to be the ruminal fluid, but may also be related to microbial processes such as chain elongation and conversion to other FAs [33].

Fatty acids are metabolised in the rumen by lipolysis, biohydrogenation, and synthesis, so the amount of fatty acids coming out of the rumen is a result of their content in the diet, and changes in profile during fermentation [14]. Jenkins (1993) and Beam et al.

(2000), after analysing rumen fat metabolism in cows and sheep and its flow to the duodenum, conclude that it corresponds to the amount of fat intake with feed [34,35]. Moate et al. (2004) reported the highest correlation coefficient ($r^2 > 0.91$) between rumen acid levels and flow into the duodenum (r2 > 0.91) for: C16:0, C18:0, C18:1t, C18:1c, C18:2, and C18:3. They also found that the estimated biohydrogenation rates of C16:1, C18:1c, C18:1t, C18:2 acids were: 39.3, 27.4, 22.8, and 87.6 %/h, respectively [36].

In our study, an increased proportion of DDGS in the substrate resulted in a higher fat level, which is also confirmed by other studies [37,38]. The effect of fermentation time on the increase in SFA levels, and the decrease in the proportion of certain unsaturated acids (C18:1n7t, C18:3n3, C18:2n6c, EPA, and UFA) in the ruminal fluid was also observed, which may be indicative of properly occurring biohydrogenation processes. In 2014, Castillo-Lopez et al. reported similar biohydrogenation for C18:3n6 acid in the rumen for TMR and DDGS-added feeds, at an average of 84.8 ± 1.0%. In contrast, the biohydrogenation of C18:2n6 acid increases in the rumen content when providing DDGS [35]. Moreover, our study showed that the level of C18:3n6 in the substrates was higher, and along with an increase in the proportion of DDGS in the substrates, it resulted in a significant and systematic decrease in this acid content. In contrast, the substrate containing only DDGS was characterised by higher levels of C18:2n6c, with higher levels of this acid in the ruminal fluid after in vitro fermentation.

Milk and dairy products, as well as meat products, are a source of fatty acids in the human diet [39–41]. Excessively high levels of SFAs, including mainly C12:0, C14:0, and C16:0 acids, adversely affect LDL-C cholesterol with detrimental effects on the human cardiovascular system [42,43]. The mammary gland of ruminants is characterised by the differential uptake of lipoprotein fractions from plasma and their transfer from diet to milk [43]. Therefore, it is important that the fatty acid profile of the ruminal fluid be characterised by high levels of long-chain unsaturated fatty acids. Feeding a diet rich in unsaturated fatty acids may increase PUFA and decrease SFA in cattle meat; however, a favourable effect on the fatty acid profile in milk and meat can also be obtained by dietary supplementation modifying rumen lipid metabolism [25,40]. In our study, the use of DDGS as a substrate resulted in increased UFA levels and decreased SFA in ruminal fluid. The resulting fatty acid profile can be considered favourable, particularly with the addition of DDGS at 15% and 20% in the dry matter of the TMR (substrates II and III, respectively). The saturated and branched milk fatty acids from C14:0 to C17:0 are mainly derived from the biosynthesis of rumen bacteria, reflecting fermentation activity [44]. The total transfer of C16:0 from diet to milk fat varies from 12% to 50%. The de novo synthesis of this acid in the mammary gland accounts for about 40% to 50% of the total amount in milk, from which it follows that the diet determines 50% of the C16:0 level in milk [45]. In our study, the use of DDGS at 10% DM in the TMR ratio (substrate I) resulted in higher levels of this acid relative to the other groups containing more DDGS. In the case of C14:0 acid in the control group, at 24 h of fermentation, the level of this acid increased with respect to groups II, III, and IV. Analysing the results of our study concerning the profile of unsaturated acids in the ruminal fluid, the most beneficial changes were observed using 15%, 20%, and 100% DDGS in the DM of the TMR (substrates II, III, and IV). Although the experiment did not include any analysis of the effect of DDGS addition on the physico-chemical characteristics of milk or meat, due to the specific level of fatty acids in DDGS, favourable changes in the fatty acid profile of milk and meat fat can be expected. This thesis is supported by the results of studies on ruminants, which reported an increase in the content of certain long-chain fatty acids, including CLA, an increase in PUFA, and a decrease in SFA in cattle products [46–52].

## 5. Conclusions

This study showed changes in the rumen in vitro fermentation profile following the introduction of maize distillers dried grains into the substrates. The higher proportion of DDGS had a favourable effect on increasing the level of unsaturated acids in the ruminal

fluid after in vitro fermentation. The inclusion of DDGS caused an increase in the proportions of C18:1n9c, C18:2n6c, 20:4n6, and EPA, but a decrease in C18:3n3 proportions in the ruminal fluid. The proportion of 15% and 20% DDGS in the dry matter of the ration was found to be most beneficial.

The results obtained may contribute to a better utilisation of the byproduct of bioethanol production and an improved fatty acid profile in the rumen content, which may result in an enhanced quality of animal products.

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## References

1. Tres, S.A.; Hennan, S.P.; Ruth, S. Authentication of dried distilled grain with solubles (DDGS) by fatty acid and volatile profiling. *LWT—Food Sci. Technol.* **2014**, *59*, 215–221. [CrossRef] [PubMed]
2. Mammi, L.M.E.; Buonaiuto, G.; Ghiaccio, F.; Cavallini, D.; Palmonari, A.; Fusaro, I.; Massa, V.; Giorgino, A.; Formigoni, A. Combined Inclusion of Former Foodstuff and Distiller Grains in Dairy Cows Ration: Effect on Milk Production, Rumen Environment, and Fiber Digestibility. *Animals* **2022**, *12*, 3519. [CrossRef] [PubMed]
3. Au, F.; McKeown, L.E.; McAllister, T.A.; Chaves, A.V. Fermentation characteristics of corn-, triticale- and wheat-based dried distillers' grains with solubles in barley-based diets determined using continuous and batch culture systems. *J. Sci. Food Agric.* **2010**, *90*, 2074–2082. [CrossRef] [PubMed]
4. Wang, K.; Zhang, J.; Tang, L.; Zhang, H.; Zhang, G.; Yang, X.; Liu, P.; Mao, Z. Establishment and assessment of a novel cleaner production process of corn grain fuel ethanol. *Bioresour. Technol.* **2013**, *148*, 453–460. [CrossRef]
5. Meyer, U.; Schwabe, A.; Dänicke, S.; Flachowsky, G. Effects of byproducts from biofuel production on the performance of growing fattening bulls. *Anim. Feed Sci. Technol.* **2010**, *161*, 132–139. [CrossRef]
6. Nuez-Orti, W.G.; Yu, P. Nutrient variation and availability of wheat DDGS, corn DDGS and blend DDGS from bioethanol plants. *J. Sci. Food Agric.* **2009**, *89*, 1754–1761. [CrossRef]
7. Srinivasan, R.; To, F.; Columbus, E. Pilot scale fiber separation from distillers dried grains with solubles (DDGS) using sieving and air classification. *Bioresour. Technol.* **2009**, *100*, 3548–3555. [CrossRef]
8. Stallings, C.C. *Distiller's Grains for Dairy Cattle and Potential Environmental Impact*; Virginia Cooperative Extension: Petersburg, VA, USA, 2009; pp. 1–3.
9. Zachwieja, A.; Kupczyński, R.; Bodarski, R.; Paczyńska, K.; Miśta, D.; Pecka, E.; Zawadzki, W.; Tumanowicz, J.; Adamski, M. DDGS uboczny produkt z wytwarzania bioetanolu—Dodatek paszowy stosowany w żywieniu przeżuwaczy. *Przemysł Chem.* **2013**, *92*, 1050–1055.
10. Abdelqader, M.M.; Oba, M. Lactation performance of dairy cows fed increasing concentrations of wheat dried distillers grains with solubles. *J. Dairy Sci.* **2012**, *95*, 3894–3904. [CrossRef]
11. Sarramone, J.P.; Gervais, R.; Benchaar, C.; Chouinarda, P.Y. Lactation performance and milk fatty acid composition of lactating dairy cows fed Camelina sativa seeds or expeller. *Anim. Feed Sci. Technol.* **2020**, *270*, 114697. [CrossRef]
12. Ranathunga, S.D.; Kalscheur, K.F.; Anderson, J.L.; Herrick, K.J. Production of dairy cows fed distillers dried grains with solubles in low- and high-forage diets. *J. Dairy Sci.* **2018**, *101*, 10886–10898. [CrossRef] [PubMed]
13. Aldai, N.; Klieve, A.V.; Dugan, M.E.R.; Kramer, J.K.G.; Ouwerkerk, D.; Aalhus, J.L.; McKinnon, J.J.; McAllister, T.A. Evaluation of rumen fatty acid hydrogenation intermediates and differences in bacterial communities after feeding wheat or corn-based dried distillers grains to feedlot cattle. *J. Anim. Sci.* **2012**, *90*, 2699–2709. [CrossRef] [PubMed]
14. Lourenco, M.; Ramos-Morales, E.; Wallace, R.J. The role of microbes in rumen lipolysis and biohydrogenation and their manipulation. *Animal* **2010**, *4*, 1008–1023. [CrossRef]
15. IZ-INRA. Standards for Cattle Nutrition. In *Standards for Ruminants Nutrition*; National Research Institute of Animal Production: Kraków, Poland, 2016; pp. 21–81. (In Polish)
16. McDougall, E.I. Studies on Ruminant Saliva. 1. The composition and output of sheep's saliva. *Biochem. J.* **1948**, *43*, 99–109. [CrossRef] [PubMed]

17. Christie, W.; William, S. *Lipid Analysis. Isolation, Separation, Identification and Structural Analysis of Lipids. The Isolation of Lipids from Tissues*; Pergamon Press: Oxford, UK, 1973; pp. 39–40.
18. Christopherson, S.W.; Glass, R.L. Preparation of milk fat methyl esters by alcoholysis in an essentially nonalcoholic solution. *J. Dairy Sci.* **1969**, *52*, 1289–1290. [CrossRef]
19. AOAC. *International Official Methods of Analysis*, 18th ed.; AOAC International: Arlington, TX, USA, 2005.
20. Holst, D.O. Holst filtration apparatus for Van Soest detergent fibre analysis. *J. AOAC* **1973**, *56*, 1352–1356. [CrossRef]
21. *Nutrient Requirements of Dairy Cattle*, 7th ed.; National Academy Press: Washington, DC, USA, 2001.
22. Horst, R.L.; Goff, J.P. Milk fever and dietary potassium. In *Proceedings of the Cornell Nutrition Conference for Feed Manufacturers*; Cornell University: Ithaca, NY, USA, 1997; pp. 181–189.
23. Gomez, K.A.; Gomez, A.A. *Statistical Procedures for Agricultural Research*, 2nd ed.; John wiley & sons: Hoboken, NJ, USA, 1984.
24. R Core Team 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing: Vienna, Austria; Available online: https://www.R-project.org/ (accessed on 20 March 2023).
25. Toral, P.G.; Monahan, F.J.; Hervás, G.; Frutos, P.; Moloney, A.P. Review: Modulating ruminal lipid metabolism to improve the fatty acid composition of meat and milk. Challenges and opportunities. *Animal* **2018**, *12*, 272–281. [CrossRef]
26. Benchaar, C.; Hassanat, F.; Gervais, R.; Chouinard, P.Y.; Julien, C.; Petit, H.V.; Massé, D.I. Effects of increasing amounts of corn dried distillers grains with solubles in dairy cow diets on methane production, ruminal fermentation, digestion, N balance, and milk production. *J. Dairy Sci.* **2013**, *96*, 2413–2427. [CrossRef]
27. Nakamura, T.; Klopfenstein, T.J.; Britton, R.A. Evaluation of acid detergent insoluble nitrogen as an indicator of protein quality in nonforage proteins. *J. Anim. Sci.* **1994**, *72*, 1043–1048. [CrossRef]
28. Patra, A.K. The effect of dietary fats on methane emissions, and its other effects on digestibility, rumen fermentation and lactation performance in cattle: A meta-analysis. *Livest. Sci.* **2013**, *155*, 244–254. [CrossRef]
29. Knapp, J.R.; Laur, G.L.; Vadas, P.A.; Weiss, W.P.; Tricarico, J.M. Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. *J. Dairy Sci.* **2014**, *97*, 3231–3261. [CrossRef] [PubMed]
30. Mišta, D.; Pecka, E.; Zachwieja, A.; Zawadzki, W.; Bodarski, R.; Paczyńska, K.; Tumanowicz, J.; Kupczyński, R.; Adamski, M. In Vitro Ruminal Fluid Fermentation as Influenced by Corn-Derived Dried Distillers' Grains with Solubles. *Folia Biol.* **2014**, *62*, 345–351. [CrossRef] [PubMed]
31. Moss, A.; Jouany, J.P.; Newbold, J. Methane production by ruminants: Its contribution to global warming. Annales de zootechnie. *INRA/EDP Sci.* **2000**, *49*, 231–253. [CrossRef]
32. Blanco, C.; Bodas, R.; Prieto, N.; Morán, L.; Andrés, S.; López, S.; Giráldez, F.J. Vegetable oil soap stocks reduce methane production and modify ruminal fermentation. *Anim. Feed Sci. Technol.* **2012**, *176*, 40–46. [CrossRef]
33. Wu, Z.; Palmquist, D.L. Synthesis and biohydrogenation of fatty acids by ruminal microorganisms in vitro. *J. Dairy Sci.* **1991**, *74*, 3035–3046. [CrossRef]
34. Jenkins, T.C. Lipid metabolism in the rumen. *J. Dairy Sci.* **1993**, *76*, 3851–3863. [CrossRef] [PubMed]
35. Beam, T.M.; Jenkins, T.C.; Moate, P.J.; Kohn, R.A.; Palmquist, R.A. Effects of amount and source of fats on the rates of lipolysis and biohydrogenation of fatty acids in ruminal contents. *J. Dairy Sci.* **2000**, *83*, 2564–2573. [CrossRef]
36. Moate, P.J.; Chalupa, W.; Jenkins, T.C.; Boston, R.C. A model to describe ruminal metabolism and intestinal absorption of long chain fatty acids. *Anim. Feed Sci. Technol.* **2004**, *112*, 79–105. [CrossRef]
37. Castillo-Lopez, E.; Ramirez, H.A.; Klopfenstein, T.J.; Anderson, C.L.; Aluthge, N.D.; Fernando, S.C.; Jenkins, T.; Kononoff, P.J. Effect of feeding dried distillers grains with solubles on ruminal biohydrogenation, intestinal fatty acid profile, and gut microbial diversity evaluated through DNA pyro-sequencing. *J. Anim. Sci.* **2014**, *92*, 33–43. [CrossRef]
38. Pecka-Kiełb, E.; Zawadzki, W.; Zachwieja, A.; Michel, O.; Mazur, M.; Mišta, D. In vitro study of the effect of corn dried distillers grains with solubles on rumen fermentation in sheep. *Pol. J. Vet. Sci.* **2015**, *18*, 751–758. [CrossRef]
39. Fusaro, I.; Cavallini, D.; Giammarco, M.; Manetta, A.C.; Martuscelli, M.; Mammi, L.M.E.; Lanzoni, L.; Formigoni, A.; Vignola, G. Oxidative Status of Marchigiana Beef Enriched in n-3 Fatty Acids and Vitamin E, Treated With a Blend of Oregano and Rosemary Essential Oils. *Front. Vet. Sci.* **2021**, *8*, 662079. [CrossRef]
40. Fusaro, I.; Cavallini, D.; Giammarco, M.; Serio, A.; Mammi, L.M.E.; De Matos Vettori, J.; Lanzoni, L.; Formigoni, A.; Vignola, G. Effect of Diet and Essential Oils on the Fatty Acid Composition, Oxidative Stability and Microbiological Profile of Marchigiana Burgers. *Antioxidants* **2022**, *11*, 827. [CrossRef]
41. Månsson, H.L. Fatty acids in bovine milk fat. *Food Nutr. Res.* **2008**, *52*, 1821. [CrossRef] [PubMed]
42. Mensink, R.P.; Zock, P.L.; Kester, A.D.; Katan, M.B. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: A meta-analysis of 60 controlled trials. *Am. J. Clin. Nutr.* **2003**, *77*, 1146–1155. [CrossRef]
43. Siri-Tarino, P.F.; Chiu, S.; Bergeron, N.; Krauss, R.M. Saturated fats versus polyunsaturated fats versus carbohydrates for cardiovascular disease prevention and treatment. *Annu. Rev. Nutr.* **2015**, *35*, 517–543. [CrossRef]
44. Offer, N.W.; Speake, B.K.; Dixon, J.; Marsden, M. Effect of fish-oil supplementation on levels of (n-3) poly-unsaturated fatty acids in the lipoprotein fractions of bovine plasma. *Anim. Sci.* **2001**, *73*, 523–531. [CrossRef]
45. Vlaeminck, B.; Fievez, V.; Cabrita, A.R.J.; Fonseca, A.J.M.; Dewhurst, R.J. Factors affecting odd- and branched-chain fatty acids in milk: A review. *Anim. Feed Sci. Technol.* **2006**, *131*, 389–417. [CrossRef]

46. Loften, J.R.; Linn, J.G.; Drackley, J.K.; Jenkins, T.C.; Soderholm, C.G.; Kertz, A.F. Invited review: Palmitic and stearic acid metabolism in lactating dairy cows. *J. Dairy Sci.* **2014**, *97*, 4661–4674. [CrossRef] [PubMed]
47. Leonardi, C.; Bertics, S.; Armentano, L.E. Effect of increasing oil from distillers grains or corn oil on lactation performance. *J. Dairy Sci.* **2005**, *88*, 2820–2827. [CrossRef]
48. Shee, C.N.; Lemenager, R.P.; Schoonmaker, J.P. Feeding dried distillers grains with solubles to lactating beef cows: Impact of excess protein and fat on cow performance, milk production and pre-weaning progeny growth. *Animal* **2016**, *10*, 55–63. [CrossRef]
49. Gaillard, C.; Sørensen, M.T.; Vestergaard, M.; Weisbjerg, M.R.; Basar, A.; Larsen, M.K.; Martinussen, H.; Kidmose, U.; Sehested, J. Effect of substituting soybean meal and canola cake with grain-based dried distillers grains with solubles as a protein source on feed intake, milk production, and milk quality in dairy cows. *J. Dairy Sci.* **2017**, *100*, 7980–7989. [CrossRef]
50. Neofytou, M.C.; Michael, C.; Constantinou, C.; Sparaggis, D.; Tzamaloukas, O. Feeding wheat dried distillers' grains with solubles increases conjugated linoleic acid and unsaturated lipids in ovine milk without adversely affecting milk yield. *J. Dairy Res.* **2021**, *88*, 128–133. [CrossRef] [PubMed]
51. Segers, J.R.; Stewart, R.L., Jr.; Lents, C.A.; Pringle, T.D.; Froetschel, M.A.; Lowe, B.K.; McKeith, R.O.; Stelzleni, A.M. Effect of long-term corn by-product feeding on beef quality, strip loin fatty acid profiles, and shelf life. *J. Anim. Sci.* **2011**, *89*, 3792–3802. [CrossRef] [PubMed]
52. Dahmer, P.L.; McDonald, F.B.; Chun, C.K.Y.; Zumbaugh, C.A.; Jones, C.K.; Crane, A.R.; Kott, T.; Lattimer, J.M.; Chao, M.D. Evaluating the impact of feeding dried distillers grains with solubles on Boer goat growth performance, meat color stability, and antioxidant capacity. *Transl. Anim. Sci.* **2022**, *9*, txac060. [CrossRef] [PubMed]