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ORIGINAL PAPER

Impact of substituting corn silage with ensiled modified corn WDGS on milk yield, composition, cow health, and production economics

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ABSTRACT. The study aimed to analyse the impact of silage from modified corn wet distillers' grains plus solubles (WDGS) in dairy cow diets on milk production, composition, animal health and economic performance. The modified WDGS were prepared as a mixture of wet distillers' grains (WDG), non-normative corn fraction (NNCF) and condensed corn distillers solubles (CCDS) in a ratio of 61:32:7 on a dry matter (DM) basis. Thirty dairy cows were divided into 3 groups (n = 10): a control group fed grass silage, corn silage, and concentrates; group W1 with 30% of corn silage DM replaced by WDGS silage; and group W2 with 65% DM replacement. Milk production, composition, and blood parameters were measured at baseline, and after 30 and 60 days. Cost and profitability analyses were also carried out. The highest milk production was recorded in group W2, while the lowest in the control group ($P \leq 0.01$) during all measurements. On day 60, group W1 showed the highest content of polyunsaturated fatty acids (PUFA) ($P \leq 0.01$), including ω-6 ($P \leq 0.05$), while group W2 had the highest ω-3 content ($P \leq 0.05$). Meanwhile, the highest concentration of plasma non-esterified fatty acids (NEFA) ($P \leq 0.05$), γ-glutamyltransferase (γ-GT) activity ($P \leq 0.05$) and malondialdehyde (MDA) content was determined in the control group ($P \leq 0.01$). Modified corn WDGS silage is a valuable feed that can replace corn silage, reduce the need for protein components in dairy cow diets, and improve the profitability of milk production.

Introduction

Biofuels, especially bio-ethanol, produced from renewable resources like corn grain have become increasingly important in recent years (Amândio et al., 2022). This production process generates various by-products including wet distillers' grains plus solubles (WDGS), condensed corn distillers solubles (CCDS) and dried distillers' grains plus solubles (DDGS). While DDGS are more commonly used in livestock nutrition, WDGS can be considered a viable feedstuff for cattle and pigs, primarily due to its economic advantages resulting from the elimination of energy-intensive drying processes. However, WDGS present certain challenges, including a relatively low dry matter (DM) content (35–45%), high water activity, and microbial instability, which consequently reduces their storage time (Rosentrater and Yang, 2021). WDGS quality can be improved by adding processed corn grains, including a non-normative corn fraction (NNCF), or co-ensiling with whole corn plants (Moyo et al., 2021;

Troyer et al., 2023). Corn WDGS typically contain 25–30% crude protein (CP) and 7–12% crude fat on a DM basis (Nuttelman et al., 2011). Their protein profile includes 15–20% rumen undegraded protein (DM basis), representing approximately 50% of total CP (MacDonald et al., 2007). While nutritionally valuable, corn WDGS are deficient in some exogenous amino acids, particularly methionine and lysine. The material serves as an excellent energy source for ruminants due to its fat content and 60% neutral detergent fibre (NDF) digestibility (Al-Suwaiegh et al., 2002; Ahern et al., 2016). Moreover, the WDGS fat composition is characterised by a rather high content of polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA). Compared to DDGS and CCDS, WDGS contains higher concentrations of bioactive compounds including carotenoids (lutein and derivatives), tocopherols (α-tocopherol), phenolic acids (ferulic, p-coumaric, caffeic), and zein proteins (Tracey et al., 2010; Yanchi, 2015; Cui et al., 2021).

Ensiled corn WDGS can effectively supplement the feed base for livestock farms, particularly those specialising in cattle husbandry. According to Kavitha et al. (2021) and Spore et al. (2021), WDGS can be incorporated into dairy cow diets at up to 35% DM without any adverse effects on milk composition and production. This by-product can partially replace traditional forages like corn silage, offering a viable alternative during periods of forage shortage. Furthermore, WDGS inclusion may reduce dependence on protein-rich concentrates in ruminant rations (Grossi et al., 2022).

Based on the presented findings, it can be safely assumed that ensiled modified corn WDGS could partially replace corn silage, which would likely reduce the need for concentrates and, consequently, lower diet costs. This substitution may additionally improve milk quality and animal health. The current study specifically evaluated modified WDGS supplemented with NNCF as a partial corn silage replacement, assessing its effects on: (1) milk production and quality, (2) health parameters, and (3) feed efficiency.

Material and methods

All cows were individually housed in a tie-stall barn with free access to water, in compliance with the Polish Council on Animal Care guidelines and the EU Directive 2010/63/EU of 22 September 2010. All procedures were approved by the 2nd Local Animal Research Ethics Committee in Warsaw (Decision No. WAW2/151/2023).

Animals and housing

The experiment was conducted over a period of 3 months. Thirty Polish Holstein-Friesian dairy cows were equally divided into three treatment groups (10 per group). The experimental animals were selected based on the analogue principle, considering factors such as age (from 2nd to 5th lactation, with the majority in the 3rd lactation), days after calving (20–60 days), milk production during the last lactation (26.7 ± 2.05 kg), and body condition score at the beginning of the study (3.2 ± 0.2). Indoor temperature was maintained at 10–15 °C and relative humidity below 80%.

Experimental feed – composition and preparation

During the experiment, the animals were fed diets supplemented with the experimental WDGS mixture. The mixture components – WDG, NNCF and CCDS were prepared accordingly at a bioethanol-production plant (Bioagra SA, Nysa, Poland). The components were blended in a ratio of 75:15:10 on a fresh matter basis, equivalent to 61:32:7 on a DM basis. The final mixture was transported to the dairy farm and ensiled in foil sleeves without any additives facilitating the ensiling process. After 10 weeks of ensiling, the WDGS mixture was incorporated into the cows' diets for a 3-month period. Prior to full implementation, a 2-week introductory period allowed for gradual adaptation, during which feed inclusion level was steadily increased.

Experimental design

The inclusion of WDGS in the diets served as the differentiating factor between the groups. The control group was fed a standard diet consisting of grass silage, corn silage, and concentrates. In the experimental groups (W1 and W2), WDGS mixture silage was included in the diets, partially replacing corn silage. In group W1, the WDGS mixture silage constituted approx. 10% of diet DM, replacing about 30% of corn silage DM in comparison to the control group. In group W2, the WDGS mixture silage constituted 20% of diet DM and replaced about 65% of corn silage DM.

The diets were formulated according to IZ PIB-INRA (2014) guidelines and prepared as total mixed rations (TMR). The nutrition value of the feeds, expressed in INRA units, including UFL (net energy feed unit for milk production), PDIN (protein digested in the small intestine when nitrogen is limiting), and PDIE (protein digested in the small intestine when energy is limiting), were calculated using INRAtion PrévAlim ver. 3.3 software.

The composition and nutritive value of the experimental diets are presented in Table 1. TMR was prepared for each group to provide 105–110% of the expected feed intake. Cows were individually fed twice daily, with 50% of the total diet offered at 8:00 and the remaining at 17:00. Daily feed refusals were collected and weighed before the morning feeding. The feed intake for each group was calculated daily as the difference between the feed offered and refused. The average feed intake per cow was determined by dividing the feed intake per group by the number of animals. For diet composition verification, weekly feed samples from each treatment group were collected, and stored at −20 °C until analysis.

Table 1. Composition and nutritive value of the experimental diets for lactating cows

Feeds and nutrients	Control	W1	W2
	Dry matter (DM), g/kg		
Wheat straw	35	35	35
Grass silage	314	344	363
Corn silage	293	200	140
WDGS silage	-	104	200
Concentrate	139	140	122
Soybean meal	56	14	11
Rapeseed meal	53	53	19
Dehydrated sugar beet	87	87	87
Feed limestone	7	7	7
Dicalcium phosphate	4	4	4
Sodium bicarbonate	5	5	5
Premix[1]	7	7	7
DM intake, kg	23.6	24.5	25.1
Nutritive value of 1 kg DM of diet			
UFL	0.94	0.94	0.95
Crude protein, g	160.9	160.4	161.2
PDIN, g	104.2	105.4	109.6
PDIE, g	92.7	95.5	99.9
PDIA, g	46	49	53
NDF, g	401.8	405.5	408.2
ADF, g	247.6	250.1	250.2
NFC, g	347.8	339.7	332.2
Crude ash, g	58	57	56
Total carotenoids, mg	63.5	75.8	83.8
Crude fat, g	31.5	37.4	42.4
SFA, g/100 g of total FA	25.1	23.5	22.4
MUFA, g/100 g of total FA	26.3	25.4	23.9
PUFA, g/100 g of total FA	43.1	44.1	45.8

Control group – standard diet based on grass silage, corn silage, and concentrates; W1 group – wet distillers' grains plus solubles (WDGS) mixture silage constituted about 10% of diet DM and replaced about 30% of corn silage DM compared to the control group; W2 group – wet distillers' grains plus solubles WDGS mixture silage constituted 20% of diet DM and replaced about 65% of corn silage DM. UFL – net energy feed unit for milk production (1 UFL = 1.7 Mcal); PDIN – protein digested in the intestines supplied by microbial protein from rumen-degraded dietary protein; PDIE – protein digested in the intestines supplied by microbial protein from rumen-fermented organic matter; PDIA – protein digested in the intestines supplied by rumen-undegraded dietary protein; NDF – neutral detergent fibre; ADF – acid detergent fibre; NFC – nonfibrous carbohydrates; SFA – saturated fatty acid; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; FA – fatty acids

Composition and nutrition values of concentrates: corn, barley, rapeseed meal, soybean meal, wheat bran, dehydrated brewers' grains, corn gluten meal, premix; UFL – 1.09; PDIN – 152 g/kg; PDIE – 126 g/kg; crude protein – 200 g/kg; NDF – 212 g/kg; crude fat – 33 g/kg; crude ash – 36 g/kg. [1] contained, %: Ca 22.4, P 3.5, Na 5.1, Mg 6.0; IU: vitamin A 1 100 000, vitamin D_3 180 000; g: vitamin E 4.0; mg: vitamin B_1 150, vitamin B_2 70, vitamin B_6 70, niacin 40 000, folic acid 70, calcium pantothenate 350, choline 7 000, Mn 4 500, Zn 8 000, Cu 1 500, I 250, Co 35; Se 50; mcg: vitamin B_{12} 1 000, biotin 3 000

Research procedures

Milk sampling

Milk production and milk composition were evaluated by collecting milk samples at the beginning of the experiment (measurement 1), after 30 days (measurement 2), and after 60 days (measurement 3). A portion of the milk collected on days 30 and 60 from each experimental group was frozen and stored at −20 °C for subsequent determination of fatty acid and carotenoid profiles.

Blood sampling

Blood samples were drawn from all cows at the same three time points as milk collections, i.e. at the beginning of the experiment, and after 30 and 60 days. The blood was collected from the jugular vein consistently between 8:00 and 10:00 using 15 ml IMPROVACUTER® lithium heparin tubes (Guangzhou Improve Medical Instruments CO., LTD, Guangzhou, PRC). Following collection, samples were immediately transported to the laboratory, where they were centrifuged at 3 000 rpm for 5 min. The resulting plasma samples was separated, frozen at −70 °C, and stored until subsequent biochemical analysis.

Feed and milk chemical analysis

Nutritional value

The chemical composition of the feeds was determined following AOAC International (2012) methods: DM content by oven-drying at 105 °C to a constant weight, crude ash by incineration at 550 °C for 6 h, crude protein (N × 6.25) using the micro-Kjeldahl technique (Kjeltec System 1026 Distilling Unit, Foss Tecator, Hilleroed, Denmark), and crude fat after petroleum ether extraction using the Soxhlet method. NDF and acid detergent fibre (ADF) contents were determined according to Van Soest et al. (1991) using an ANKOM200 fibre analyser (ANKOM Technology, Macedon, USA).

The analysis of milk composition was performed on fresh milk samples. Constituents, including protein, fat, urea, and lactose, were determined using Fourier-transform infrared spectroscopy (FTIR) according to PN-ISO 9622:2015-09 with a Milkoscan FT+ analyser (FossElectric, Hilleroed, Denmark). Somatic cell count (SCC) was determined following PN-EN ISO 13366-2:2007, using a Fossomatic FC (Bentley Instruments Inc., Chaska, USA).

Fatty acid composition analysis

The fatty acid composition in extracted milk fat samples was analysed using gas chromatography with flame ionization detection (GC/FID) according to PN-EN ISO 12966-1:2015 + AC:2015-06, PN-EN ISO 12966-2:2017-05 pkt. 5.2, and PN-EN ISO 12966-4:2015-07 standards. Prior to analysis, fatty acids were converted to methyl esters via alkaline transesterification. The analysis was performed using a Bruker 456 SCION-GC system (Bruker Corporation, Billerica, USA) equipped with a 100 m × 0.25 mm ID, 0.20 µm Restek Rt-2560 capillary column (Restek, Lisses, France). The following fatty acid groups were determined: saturated fatty acids (SFA) – C4:0, C6:0, C8:0, C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C 18:0, C20:0, C21:0, and C23:0; unsaturated fatty acids (UFA) – TFA, MUFA, and PUFA; trans fatty acids (TFA) – C18:1t, C18:2n6tt, C18:2n6ct, and C18:3n3t; monounsaturated fatty acids (MUFA) – C14:1, C16:1, and C17:1; polyunsaturated fatty acids (PUFA) – PUFA ω-3, and PUFA ω-6; PUFA ω-3 – C18:1n9c, C18:1n7c, C18:3n3; and PUFA ω-6 – C18:2n6c. Based on the fatty acid groupings, nutritional indices were calculated, including the atherogenicity index (AI), thrombogenicity index (TI), and the ratio of saturated to polyunsaturated fatty acids (S/P) according to Ulbricht and Southgate (1991), as well as the ratio of hypocholesterolaemic to hypercholesterolaemic fatty acids (DFA/OFA) following Fernández et al. (2007).

Carotenoid profile

The quantitative and qualitative determinations of carotenoids in milk and diets were performed using a Dionex high-performance liquid chromatography system (Polygen Polska, Gliwice, Poland) equipped with a CoulArray electrochemical detector (ESA Inc.; Polygen Polska, Gliwice, Poland). Separation was conducted using a Hypersil BDS 150 4.6 mm, 5 µm column (Sigma-Aldrich, Saint Louis, USA,) at a mobile phase flow rate of 1.2 ml/min. The mobile phase consisted of a methanol and isopropanol mixture (98:2). For electrochemical detection, 4 electrodes with potentials of 400, 500, 600, and 750 mV were utilised. Carotenoid identification was based on retention times of authentic standards, chromatographic peak areas, and the ratio of peak areas between the dominant electrode and adjacent electrodes.

Blood plasma biochemical parameters

Dairy cows' plasma samples were analysed for total cholesterol (TCHO) (Cat. No. 257914, FUJIFILM Corporation, Minato, Japan), urea nitrogen (BUN) (Cat. No. 370314, FUJIFILM Corporation, Minato, Japan), total protein (TP) (Cat. No. 473014, FUJIFILM Corporation, Minato, Japan), calcium (Ca) (Cat. No. 600346, FUJIFILM Corporation, Minato, Japan), glucose (GLU) (Cat. No. 118714, FUJIFILM Corporation, Minato, Japan), inorganic phosphorus (IP) (Cat. No. 600340, FUJIFILM Corporation, Minato, Japan), albumin (ALB) and globulin (GLOB) (Cat. No. 158414, FUJIFILM Corporation, Minato, Japan), aspartate aminotransferase activity (AST) activity (Cat. No. 462014, FUJIFILM Corporation, Minato, Japan) using a DRI-CHEM NX500 automated biochemistry analyser (Fujifilm Healthcare, Chiba, Japan). The concentrations of β-hydroxybutyric acid (BHBA), non-esterified fatty acids (NEFA), and gamma-glutamyl transpeptidase (γ-GT) were measured using a BS800M chemistry analyser (Mindray Medical, Warsaw, Poland). Plasma lipid peroxidation and oxidative status were determined using the QuantiChrom™ TBARS Assay Kit (BioAssay Systems; Hayward, CA, USA). The TBARS assay uses thiobarbituric acid as a reagent to detect lipid decomposition and peroxidation products, such as malondialdehyde (MDA). The analysis was performed according to the manufacturer's protocol. First, a 100 µl plasma from each sample was pipetted out into empty Eppendorf tubes, to which 200 µl of 10% solution of trichloroacetic acid was added and incubated on ice for 5 min. Afterwards, the samples were centrifuged at 14 000 rpm for 5 min. Next, a 200 µl aliquot of the supernatants was transferred into new vials, and 200 µl of TBA reagent was added. The vials were incubated at 100 °C for 60 min. Following incubation, absorbance was measured at 535 nm using an INFINITE M NAN microplate reader (TECAN, Männedorf, Switzerland). Plasma MDA concentration (µmol/l) was determined by first subtracting blank absorbance values from all readings, then calculating the standard curve slope, and finally applying the formula:

$$\text{TBARS (µmol/l MDA equiv.)} = \frac{(\text{Absorbance of}_{\text{Sample}} - \text{Absorbance of}_{\text{Blank}}) \times \text{Dilution factor}}{\text{Slope}}$$

Effect of diet composition and milk production on economic indices

Mean values of milk yield, feed composition, feed intake, and milk and feed prices were used to calculate economic indices expressed in Euros (EUR), based on prices from the Central Statistical Office of Poland (GUS, 2023), and the current stock exchange values of feed components (KPODR, 2023). Since all data used were mean values, the economic indices were not subjected to statistical analysis. The following economic indices were calculated: average milk income, milk-to-feed price ratio (MFPR), income over feed cost (IOFC), and income equals feed cost (IEFC). The IOFC, a key profitability indicator for dairy farm decision-making, was calculated according to Kung and Huber (1983), as cited in Atzori et al. (2021). The IEFC determining the milk production needed to cover feeding costs, was calculated using the formula of Pepin (2009), as cited in Atzori et al. (2021). The MFPR assesses the profitability of dairy production through feed costs, milk price, and milk quality. It was calculated using the formula by Wolf (2010), as referenced in Atzori et al. (2021).

Statistical analysis

Statistical analyses were conducted using Statistica ver. 13.3 (Statsoft Polska, Cracow, Poland). Data normality was assessed using the ShapiroWilk test, while homogeneity of variances was verified with Levene's test. For data meeting parametric assumptions, one-way analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) were performed. Results are presented as means ± standard error of the mean (SEM), with group effects indicated by *P*-values. Significance thresholds were set at $P < 0.05$ (significant) and $P < 0.01$ (highly significant). Differences between groups were analysed using HSD Tukey's test. MANOVA was applied to assess animal performance, milk composition, and plasma biochemistry, while ANOVA was used to assess fatty acid and carotenoid profiles.

Results

Milk production and composition

The supplementation of diets with WDGS significantly affected milk production, with groups W1 and W2 showing higher yields compared to the control group ($P < 0.01$; Table 2). While milk composition parameters (fat, protein, urea, and lactose) showed no significant differences between groups, SCC was significantly elevated in group W1 relative to both control and group W2 ($P < 0.01$) Temporal analysis revealed that milk yield was highest at the first measurement point, decreased at the second measurement on day 30, and lowest at the third measurement on day 60 ($P < 0.01$). Milk composition showed time-dependent changes, with protein content and SCC increasing progressively throughout the experiment ($P < 0.05$). Conversely, milk urea and lactose concentrations decreased significantly over time ($P < 0.01$; Table 2).

Table 2. Effects of dietary treatments and measurement time on milk production and composition

Item	Treatment			SEM	*P*-value	Measurement			*P*-value	Mea x Tre *P*-value
	Control	W1	W2			1st	2nd	3rd		
Milk yield, kg	27.75^{A}	35.17^{B}	34.91^{B}	0.6278	<0.001	35.44^{C}	33.45^{B}	28.94^{A}	<0.001	0.004
Fat, %	4.83	4.44	4.67	0.069	0.060	4.44	4.73	4.79	0.075	0.741
Protein, %	3.86	3.64	3.86	0.046	0.079	3.63^{a}	3.91^{b}	3.82^{ab}	0.042	0.618
Urea, mg/l	243.89	250.00	238.21	4.601	0.507	257.26^{B}	255.07^{B}	219.78^{A}	<0.001	0.582
SCC, 10^3 cells/ml	145^{A}	181^{B}	144^{A}	3.968	<0.001	139^{A}	165^{B}	167^{B}	<0.001	0.257
Lactose, %	4.7	4.8	4.74	0.025	0.255	4.83^{B}	4.81^{B}	4.60^{A}	<0.001	0.992
Fat-to-protein ratio	1.25	1.22	1.22	0.016	0.633	1.22	1.21	1.26	0.466	0.882

Control group – standard diet based on grass silage, corn silage, and concentrates; W1 group – wet distillers' grains plus solubles (WDGS) mixture silage constituted about 10% of diet dry matter (DM) and replaced about 30% of corn silage DM compared to the control group; W2 group – WDGS mixture silage constituted 20% of diet DM and replaced about 65% of corn silage DM; SEM – standard error of the mean, SCC – somatic cells score. ^{abc} means in the same row with different superscripts are significantly different at $P < 0.05$; ^{ABC} means in the same row with different superscripts are significantly different at $P < 0.01$

Fatty acid profile

The inclusion of WDGS in the diets influenced the fatty acid profile on days 30 and 60 of the experiment.

On day 30 (Table 3), significant changes were observed only in the C17:0 content and the ω-6/ω-3 ratio. Both groups W1 and W2 showed significantly reduced concentration of margaric acid compared to the control ($P < 0.01$). The ω-6/ω-3 ratio was

Table 3. Effects of dietary treatments on the fatty acid profile of milk fat on day 30 of the experiment (g/100 g of fatty acids) and values of health-promoting indices

Item	Treatment			SEM	*P*-value
	Control	W1	W2		
SFA	66.78	65.52	65.25	0.527	0.476
C4:0	1.91	1.93	1.95	0.036	0.900
C6:0	1.56	1.57	1.54	0.027	0.918
C8:0	1.12	1.12	1.06	0.022	0.497
C10:0	2.94	2.81	2.63	0.078	0.266
C12:0	3.81	3.43	3.22	0.135	0.200
C13:0	0.11	0.11	0.10	0.003	0.104
C14:0	12.09	11.39	11.27	0.193	0.171
C15:0	1.31	1.24	1.20	0.023	0.189
C16:0	30.99	29.76	29.64	0.523	0.534
C17:0	0.74^{B}	0.66^{A}	0.69^{A}	0.011	<0.001
C18:0	9.30	10.51	11.02	0.402	0.210
C20:0	0.16	0.16	0.17	0.008	0.851
C21:0	0.53	0.64	0.58	0.023	0.149
C23:0	0.19	0.18	0.17	0.004	0.190
UFA	27.65	28.94	29.22	0.538	0.475
TFA	2.24	2.83	2.69	0.111	0.069
C18:1t	1.62	2.15	2.02	0.096	0.056
C18:2n6tt	0.18	0.20	0.20	0.008	0.290
C18:2n6ct	0.31	0.33	0.33	0.008	0.568
C18:3n3t	0.13	0.15	0.14	0.004	0.515
MUFA	23.39	23.72	24.30	0.405	0.674
C14:1	1.24	1.19	1.18	0.055	0.915
C16:1	2.18	1.96	1.97	0.067	0.360
C17:1	0.20	0.17	0.19	0.005	0.163
PUFA	2.02	2.39	2.23	0.088	0.242
ω-3	0.28	0.26	0.28	0.007	0.475
C18:1n9c	19.36	19.96	20.56	0.438	0.564
C18:1n7c	0.42	0.40	0.39	0.012	0,574
C18:3n3	0.28	0.26	0.28	0.007	0.475
ω-6	1.74	2.13	1.95	0.085	0.177
C18:2n6c	1.70	2.04	1.91	0.082	0.238
SFA/MUFA	2.88	2.77	2.70	0.068	0.566
SFA/UFA	27.65	28.94	29.22	0.538	0.475
SFA/TFA	30.91	23.76	24.59	1.376	0.057
SFA/PUFA	34.54	28.50	29.37	1.623	0.274
MUFA/PUFA	11.99	10.16	10.90	0.441	0.249
ω-6/ω-3	6.30^{a}	8.20^{b}	7.11^{ab}	0.296	0.020
AI	3.16	2.90	2.83	0.093	0.326
TI	3.90	3.76	3.70	0.089	0.682
S/P	2.08	1.99	1.97	0.048	0.633
DFA/OFA	0.56	0.61	0.63	0.023	0.474

Control group – standard diet based on grass silage, corn silage, and concentrates; W1 group – wet distillers' grains plus solubles (WDGS) mixture silage constituted about 10% of diet dry matter (DM) and replaced about 30% of corn silage DM compared to the control group; W2 group – WDGS mixture silage constituted 20% of diet DM and replaced about 65% of corn silage DM. SFA – saturated fatty acids, UFA – unsaturated fatty acids, TFA – trans fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, ω-3 – omega 3 fatty acids, ω-6 – omega 6 fatty acids, C4:0 – butyric acid, C6:0 – caproic acid, C8:0 – caprylic acid, C10:0 – capric acid, C12:0 – lauric acid, C13:0 – tridecylic acid, C14:0 – myristic acid, C15:0 – pentadecylic acid, C16:0 – palmitic acid, C17:0 – margaric acid, C18:0 –stearic acid, C20:0 – arachidic acid, C21:0 – heneicosylic acid, C23:0 – tricosylic acid, C18:1t – elaidic acid, C18:2n6tt – linolelaidic acid, C18:2n6ct – linoleic acid, C18:3n3t – trans-linolenic acid, C14:1 – myristoleic acid, C16:1 – palmitoleic acid, C17:1 – heptadecenoic acid, C18:1n9c – oleic acid, C18:1n7c – vaccenic acid, C18:3n3 – α-linolenic acid, C18:2n6c – linoleic acid, AI – atherogenicity index, TI – thrombogenicity index, S/P – saturation, DFA/OFA – hypocholesterolaemic fatty acids to the hypercholesterolemic fatty acids; SEM – standard error of the mean. ^{abc} means in the same row with different superscripts are significantly different at $P < 0.05$; ^{ABC} means in the same row with different superscripts are significantly different at $P < 0.01$

Table 4. Effects of dietary treatments on the fatty acid profile of milk fat on day 60 of the experiment (g/100 g of fatty acids) and values of health-promoting indices

Item	Treatment			SEM	*P*-value
	Control	W1	W2		
SFA	67.64	66.12	66.95	0.583	0.595
C4:0	1.86	1.92	1.93	0.030	0.594
C6:0	1.56	1.58	1.58	0.028	0.947
C8:0	1.12	1.11	1.11	0.025	0.980
C10:0	2.90	2.80	2.83	0.086	0.885
C12:0	3.76	3.47	3.57	0.113	0.595
C13:0	0.11	0.11	0.11	0.004	0.756
C14:0	12.21	11.64	11.64	0.186	0.369
C15:0	1.29	1.25	1.34	0.022	0.243
C16:0	33.71	32.02	31.29	0.514	0.141
C17:0	0.71	0.66	0.72	0.011	0.060
C18:0	7.53	8.64	9.95	0.433	0.065
C20:0	0.13	0.15	0.17	0.007	0.081
C21:0	0.57	0.60	0.51	0.020	0.184
C23:0	0.17	0.19	0.18	0.004	0.148
UFA	26.78	28.31	27.50	0.595	0.601
TFA	2.30	2.56	2.53	0.062	0.162
C18:1t	1.59	1.83	1.82	0.055	0.131
C18:2n6tt	0.21	0.24	0.21	0.011	0.442
C18:2n6ct	0.34	0.35	0.36	0.010	0.857
C18:3n3t	0.15	0.14	0.14	0.006	0.830
MUFA	22.70	23.60	23.00	0.560	0.820
C14:1	1.57	1.28	1.22	0.071	0.094
C16:1	2.44	2.35	2.07	0.129	0.498
C17: 1	0.20	0.19	0.19	0.007	0.830
PUFA	1.78^{A}	2.15^{B}	1.98^{AB}	0.053	0.008
ω-3	0.24^{a}	0.27^{ab}	0.28^{b}	0.006	0.015
C18:1n9c	18.09	19.34	19.10	0.477	0.556
C18:1n7c	0.37	0.41	0.40	0.012	0.276
C18:3n3	0.24^{a}	0.27^{ab}	0.28^{b}	0.006	0.015
ω-6	1.55^{a}	1.89^{b}	1.70^{ab}	0.050	0.011
C18:2n6c	1.50^{a}	1.81^{b}	1.66^{ab}	0.048	0.018
SFA/UFA	2.56	2.34	2.47	0.074	0.521
SFA/TFA	29.64	25.97	26.93	0.823	0.172
SFA/MUFA	3.03	2.81	2.96	0.097	0.665
SFA/PUFA	38.33^{B}	30.87^{A}	34.13^{AB}	1.087	0.009
MUFA/PUFA	12.92	10.98	11.70	0.442	0.200
ω-6/ω-3	6.55	7.05	6.19	0.194	0.194
AI	3.42	3.06	3.17	0.102	0.370
TI	4.17	3.83	4.02	0.119	0.535
S/P	2.22	2.04	2.15	0.064	0.548
DFA/OFA	0.48	0.55	0.55	0.019	0.237

Control group – standard diet based on grass silage, corn silage, and concentrates; W1 group – wet distillers' grains plus solubles (WDGS) mixture silage constituted about 10% of diet dry matter (DM) and replaced about 30% of corn silage DM compared to the control group; W2 group – WDGS mixture silage constituted 20% of diet DM and replaced about 65% of corn silage DM. SFA – saturated fatty acids, UFA – unsaturated fatty acids, TFA – trans fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, ω-3 – omega 3 fatty acids, ω-6 – omega 6 fatty acids, C4:0 – butyric acid, C6:0 – caproic acid, C8:0 – caprylic acid, C10:0 – capric acid, C12:0 – lauric acid, C13:0 – tridecylic acid, C14:0 – myristic acidm C15:0 – pentadecylic acid, C16:0 – palmitic acid, C17:0 – margaric acid, C18:0 – stearic acid, C20:0 – arachidic acid, C21:0 – heneicosylic acid, C23:0 – tricosylic acid, C18:1t – elaidic acid, C18:2n6tt – linolelaidic acid; C18:2n6ct – Linoleic acid; C18:3n3t – trans-Linolenic acid; C14:1 – Myristoleic acid; C16:1 – Palmitoleic acid; C17:1 – heptadecenoic acid; C18:1n9c – oleic acid, C18:1n7c – vaccenic acid, C18:3n3 – α-linolenic acid, C18:2n6c – linoleic acid; AI – atherogenicity index, TI – thrombogenicity index, S/P – saturation, DFA/OFA – hypocholesterolaemic fatty acids to the hypercholesterolemic fatty acids; SEM – standard error of the mean. ^{abc} means in the same row with different superscripts are significantly different at $P < 0.05$; ^{ABC} means in the same row with different superscripts are significantly different at $P < 0.01$

significantly lower in the control group compared to group W1 ($P < 0.05$; Table 3).

On day 60 of the experiment, the experimental diets influenced the contents of milk PUFAs, ω-3, α-linolenic acid (C18:3n3), ω-6, linoleic acid (C18:2n6c), and the SFA/PUFA ratio. The highest content of PUFA was recorded in milk from group W1, while the lowest values were observed in the

control group ($P < 0.01$). A similar trend was noted for ω-6 and C18:2n6c contents, with group W1 showing the highest levels and the control group the lowest ($P < 0.05$). Conversely, the highest content of ω-3 and C18:3n3 was found for group W2, while the lowest values were detected in the control group ($P < 0.05$). The control group displayed the highest SFA/PUFA ratio, while the lowest ratio was observed in group W1 ($P < 0.01$) (Table 4).

Carotenoid profile

The carotenoid composition of milk (β-carotene, lutein, and zeaxanthin) on days 30 and 60 of the experiment is presented in Table 5. No significant differences were observed between groups at either time point ($P > 0.05$). However, a trend toward higher β-carotene, lutein, and zeaxanthin concentrations was observed in WDGS-supplemented groups (W1 and W2) compared to the control.

Table 5. Effects of dietary treatments on the carotenoid profile of milk on day 30 and 60 of the experiment

Measurement	Item	Treatment			SEM	*P*-value
		Control	W1	W2		
day 30	β-carotene, µg/ml	0.33	0.36	0.43	0.025	0.251
	Lutein, µg/ml	0.03	0.06	0.06	0.004	0.752
	Zeaxanthin, µg/ml	0.03	0.04	0.04	0.001	0.283
day 60	β-carotene, µg/ml	0.37	0.36	0.50	0.034	0.171
	Lutein, µg/ml	0.02	0.08	0.08	0.004	0.688
	Zeaxanthin, µg/ml	0.04	0.04	0.04	0.001	0.711

Control group – standard diet based on grass silage, corn silage, and concentrates; W1 group – wet distillers' grains plus solubles (WDGS) WDGS mixture silage constituted about 10% of diet dry matter (DM) and replaced about 30% of corn silage DM compared to the control group; W2 group – WDGS mixture silage constituted 20% of diet DM and replaced about 65% of corn silage DM; SEM – standard error of the mean; $P > 0.05$

Biochemical parameters of blood plasma

Biochemical parameters of blood plasma samples were analysed at three time points during the experiment (Table 6). A total of 17 parameters were evaluated, although only a subset showed significant differences between the groups.

Compared to the control group, BUN levels were significantly higher in group W1 ($P < 0.01$). Conversely, γ-GT activity decreased with increasing dietary WDGS concentration, with the highest levels observed in the control group and the lowest in group W2 ($P < 0.05$). Furthermore, NEFA and MDA levels were significantly lower in both treatments W1 and W2 compared to the control group ($P < 0.01$).

When examining temporal changes in biochemical parameters, significant differences were found for BUN, GLU, Ca, BHBA, and NEFA levels. BUN concentration reached its maximum at the second measurement time point, while GLU and Ca levels showed a gradual increase throughout the experimental period. BHBA and NEFA levels, on the other hand, decreased progressively from the first to the third measurement ($P < 0.01$).

Table 6. Effects of dietary treatments on biochemical parameters in cows' blood plasma

Item	Treatment			SEM	P-value	Measurement			*P*-value	Mea X Tre *P*-value
	Control	W1	W2			1st	2nd	3rd		
TCHO, mg/dl	189.76	187.52	190.71	4.562	0.959	189.57	199.14	179.29	0.228	0.626
BUN, mg/dl	9.25^{A}	11.40^{B}	10.25^{AB}	0.300	<0.001	9.23^{A}	12.53^{B}	9.14^{A}	<0.001	0.442
ALB, g/dl	3.34	3.40	3.36	0.042	0.859	3.38	3.38	3.33	0.849	0.260
TP, mg/dl	7.59	7.37	7.64	0.112	0.600	7.55	7.38	7.68	0.583	0.782
AST, U/l	96.00	89.52	84.62	2.822	0.276	86.52	89.00	94.62	0.503	0.760
GLU, mg/dl	57.86	59.52	59.62	1.280	0.802	53.43^{A}	59.48^{AB}	64.1^{B}	0.003	0.817
IP, mg/dl	6.03	5.67	5.99	0.092	0.258	5.91	5.84	5.94	0.902	0.971
Ca, mg/dl	6.69	6.61	6.57	0.368	0.898	4.44^{A}	4.87^{A}	10.56^{B}	<0.001	0.971
GLOB, g/dl	4.27	3.99	4.30	0.096	0.383	4.20	4.00	4.35	0.356	0.841
ALB/GLOB	0.82	0.87	0.81	0.018	0.441	0.85	0.87	0.78	0.097	0.802
γ-GT, U/l	33.54^{b}	32.82^{ab}	27.57^{a}	0.919	0.017	31.31	29.77	32.86	0.377	0.968
BHBA, mmol/l	0.73	0.77	0.75	0.018	0.657	0.75^{ab}	0.81^{b}	0.69^{a}	0.040	0.436
NEFA, mmol/l	0.11^{b}	0.09^{a}	0.09^{a}	0.003	0.031	0.11^{C}	0.10^{B}	0.08^{A}	<0.001	0.765
MDA, µmol/l	0.63^{B}	0.59^{B}	0.51^{A}	0.013	<0.001	0.57	0.55	0.60	0.210	0.343

Control group – standard diet based on grass silage, corn silage, and concentrates; W1 group – wet distillers' grains plus solubles (WDGS) mixture silage constituted about 10% of diet dry matter (DM) and replaced about 30% of corn silage DM compared to the control group; W2 group – WDGS mixture silage constituted 20% of diet DM and replaced about 65% of corn silage DM. TCHO – cholesterol, BUN – blood urea nitrogen, ALB – albumins, TP – total protein; AST – aspartate aminotransferase, GLU – glucose, IP – inorganic phospohorus, Ca – calcium, GLOB – globulins, ALB/GLOB – albumin to globulin ratio, γ-GT – γ-glutamyltransferase, BHBA – β-hydroxybutyric acid, NEFA – non-esterified fatty acids; MDA – malondialdehyde; SEM – standard error of the mean. ^{abc} means in the same row with different superscripts are significantly different at $P < 0.05$; ^{ABC} means in the same row with different superscripts are significantly different at $P < 0.01$

Table 7. Effects of dietary treatments on economic indices of milk production

Item	Treatment		
	Control	W1	W2
Percentage of concentrates in diet DM, %	34.3	30.0	24.5
Percentage of roughage in diet DM, %	64.1	68.4	73.9
Average daily cost of feeding, EUR/day per cow	8.18	7.77	7.58
Average daily cost of feeding – roughage, EUR/day per cow	4.12	4.37	4.75
Average daily cost of feeding – concentrate, EUR/day per cow	3.68	3.02	2.44
Average daily milk yield, kg/d per cow	27.8	35.2	34.9
Average milk income (yield x price), EUR/day per cow	13.68	17.34	17.21
Milk-to-feed price ratio (MFPR)	1.7	2.2	2.3
Income over feed cost (IOFC), EUR/day per cow	5.50	9.57	9.63
Income equals feed cost (IEFC), kg/day per cow	0.60	0.45	0.44

Control group – standard diet based on grass silage, corn silage, and concentrates; W1 group – wet distillers' grains plus solubles (WDGS) mixture silage constituted about 10% of diet dry matter (DM) and replaced about 30% of corn silage DM compared to the control group; W2 group – WDGS mixture silage constituted 20% of diet DM and replaced about 65% of corn silage

Effect of diet composition and milk production on economic indices

The economic analysis of milk production is presented in Table 7. The inclusion of WDGS into the diets reduced concentrate requirements, resulting in lower average daily feeding costs for groups W1 and W2 compared to the control. Moreover, the average daily milk yield was higher for both groups fed WDGS, as evidenced by the MFPR and IOFC. Conversely, the control group showed the highest IEFC, indicating the milk production costs were lower in the WDGS-supplemented groups. These results demonstrate that dietary WDGS inclusion improved the economic efficiency of milk production.

Discussion

Distillers' grains are widely utilised in ruminant nutrition (Anderson et al., 2006; Nuttelman et al., 2011). Of all bio-ethanol by-products, the DDGS remain the most popular and readily available on the market. While this is particularly true in the EU and Poland, corn WDGS continues to be extensively used and valued as a feed source for cattle, especially in the USA. This presents an opportunity for EU and Polish farms located near bioethanol plants where corn WDGS could be particularly advantageous for farms near bioethanol plants. The present study developed an enhanced corn WDG feed by supplementing CCDS and NNCF, resulting in modified chemical composition. The data demonstrate that this formulation increased DM and crude fibre content by 20 and 44%, respectively, while reducing crude fat content by 35%, without altering protein levels. Research has shown that corn WDGS can be safely incorporated into ruminant diets at levels not exceeding 35% DM (Kavitha et al., 2021; Spore et al., 2021). This study used ensiled modified corn WDGS at dietary inclusion rates of 10 and 20% DM, replacing approx. 30% DM and 65% DM of corn silage in groups W1 and W2, respectively. The inclusion enabled effective replacement of concentrated feed components in the cows' rations. Specifically, WDGS supplementation reduced the concentrate proportion by 4.3% in group W1 and 9.8% in group W2, while simultaneously decreasing the combined inclusion of soybean and rapeseed meals by 39% and 74%, respectively.

The groups receiving WDGS in the diets achieved significantly higher milk production compared to the control group. Importantly, all experimental diets were carefully formulated to maintain isoenergetic and isoprotein balance with similar NDF and ADF contents, ensuring that the observed production differences could be specifically attributed to WDGS supplementation. Taken collectively, these findings suggest that WDGS may have a positive effect on ruminal function and digestion. The high NDF digestibility of corn WDGS, as noted by Al-Suwaiegh et al. (2002), may be an important factor in improving diet utilisation and digestion, thereby contributing to increased milk production. Another key factor improving these parameters could be the higher concentration of bypass protein in corn distillers' grains. These findings are supported by the studies of Anderson et al. (2006) and Mammi et al. (2022), who similarly observed that WDGS supplementation positively influenced milk yield and overall production performance in dairy cows. Conversely, according to Nešić et al. (2023), milk yields remained unchanged and unaffected by WDGS addition. The introduction of WDGS to the cows' diets in the present study did not affect milk composition, as the fat and protein content remained unchanged between the experimental groups, which was consistent with previous studies of Anderson et al. (2006), Mammi et al. (2022), and Nešić et al. (2023). Furthermore, the fat-to-protein ratio remained within the optimal 1.2–1.4 range in the current experimental groups, suggesting proper

rumen function and absence of metabolic disorders like acidosis or ketosis, despite the dietary modifications (Atalay, 2019). Plasma metabolite analysis revealed that BHBA concentrations remained unaffected by WDGS supplementation and below the 1 mmol/l threshold, while NEFA levels, although showing treatment differences, stayed beneath the 0.7 mmol/l reference level (Krempasky et al., 2014). These metabolic parameters collectively demonstrate that all experimental diets were nutritionally balanced, maintained adequate DM intake, and avoided metabolic disorders such as acidosis or ketosis. Even though WDGS addition did not alter milk urea content, it significantly increased plasma urea levels in group W1 compared to controls. Both parameters serve as important indicators of protein utilisation by the animal, which is influenced by dietary protein content and fermentable energy availability from non-structural carbohydrates (Tshuma et al., 2019). Despite the elevated plasma urea in the W1 group compared to the control group, these values remained within the acceptable reference range of 6–19 mg/dl (Rhoads et al., 2006). Our results also showed that milk production decreased over time, while milk fat content remained unchanged. Additionally, significant temporal differences were observed for milk protein and urea concentrations. These parameters, however, remained within acceptable reference values, further corroborating that the cows were provided with well-balanced diets (Januś and Stanek, 2017). The determination of antioxidant activity was performed by the analyses of plasma γ-GT activity and MDA content. The former is an antioxidant enzyme, whose altered activity potentially indicates hepatic or renal detoxification impairment. The highest concentration of this enzyme was found in the control group, while group W2 had the lowest levels. MDA is a marker of lipid peroxidation, whose concentrations followed a similar patter, with control and W1 groups demonstrating higher values than W2. Notably, the relationship between γ-GT activity and MDA concentration suggests a potential influence of γ-GT on MDA in blood plasma. This aligns with findings by Saleh (2008), who reported that increased MDA concentrations and lipid peroxidation were strongly associated with decreased concentrations and activities of antioxidant enzymes. Moreover, the γ-GT and MDA values in this study were within the acceptable reference ranges, consistent with those reported by Premi et al. (2021) for γ-GT, and by Ventsova and Safonov (2021) for MDA.

The addition of WDGS to the diets significantly increased the levels of nutritionally valuable unsaturated fatty acids. By week 8 of the experiment, groups W1 and W2 showed increased concentrations of PUFA, including both ω-6 and ω-3 fractions, with the ω-6/ω-3 ratio rising from week 4 onward. It should be noted that these changes reflected specific fatty acid modifications: α-linolenic acid (C18:3n3) was increased in W1, while linoleic acid (C18:2n6c) was elevated in W2 during the final measurement, while decreased margaric acid (C17:0) levels were observed during the 2nd measurement. The inclusion of WDGS in dairy rations significantly improved the content of nutritionally valuable fatty acids in milk, particularly C18:3 (α-linolenic acid) and C18:2 (linoleic acid), which are especially beneficial for consumers. This increase in PUFA and ω-6 fatty acids reflects the composition of WDGS, which is naturally rich in oleic and linoleic acids (Wilson et al., 2021). Importantly, despite these changes in fatty acid profile, key health-promoting indices of milk fat (AI, TI, S/P, and DFA/OFA) remained unaffected by WDGS supplementation. While corn distillers' grains are considered a rich source of carotenoids (Tracey et al., 2010), the addition of ensiled corn WDGS in this study did not significantly affect the carotenoid composition of milk. Despite the lack of statistical differences, a trend toward higher carotenoid concentrations was observed in the WDGS-fed groups. These findings demonstrate that partial substitution of corn silage with ensiled corn WDGS can be a viable option that preserves the concentration of bioactive compounds in milk.

From a milk producer's perspective, profitability is primarily determined by feeding costs, followed by other milk production expenses. The addition of WDGS positively impacted several economic indicators of milk production, including MFPR, IOFC, and IEFC. MFPR, a parameter that integrates feed costs, milk price, and quality (Atzori et al., 2021), exceeded the 1.3 profitability threshold in all groups, with WDGS-supplemented groups (W1 and W2) showing particularly favourable values. IOFC, the primary profitability metric for dairy operations, was substantially higher in both WDGS groups compared to controls, reflecting greater financial returns after accounting for feed expenses. Similarly, the IEFC index showed enhanced production efficiency in the treated groups, as evidenced by its lower values indicating less milk required to cover feeding costs (Atzori et al., 2021).

Conclusions

The use of modified corn wet distillers' grains plus solubles (WDGS) silage in dairy cow diets has demonstrated its potential as a viable alternative to corn silage, effectively reducing the reliance on protein supplements. When incorporated at 10 to 20% of diet dry matter, WDGS significantly improved milk production, while maintaining standard concentrations of milk components such as protein, fat, lactose or carotenoids. Importantly, the polyunsaturated fatty acid content in milk increased substantially, enhancing its nutritional value, despite no significant changes in total fat percentage. This improvement in fatty acid profile appears related to both the increased fibre digestibility and the favourable fatty acid composition characteristic of corn WDGS. The inclusion of WDGS in dairy rations maintained animal health and metabolic function, with key indicators, including non-esterified fatty acids and γ-GT, showing more favourable values compared to the control group. The improved income over feed cost and income equals feed cost indices – resulting from both higher milk yield and reduced feeding costs – demonstrated that partial replacement of corn silage with modified corn WDGS silage represents a profitable strategy for dairy farmers.

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Conflicts of interest

The Authors declare that there is no conflict of interest.

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