

Article

Responses of Lactation, Rumen Fermentation and Blood Biochemical Parameters with Increasing Dietary Inulin Supplementation in Mid-Lactation Dairy Cows

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Abstract: Effects of dietary supplementation of inulin in dairy cows were investigated in this study. Thirty-six mid-lactation Holstein dairy cows were randomly divided into six groups with six cows per group and offered a total mixed ration supplemented with 0 (control), 50, 150, 200, 250 and 350 g/d inulin per head, respectively. The animals were pre-fed for 2 weeks before rumen fluid, milk and blood samples were collected weekly for 3 weeks. With increasing inulin doses, milk yield ($p < 0.01$) and milk fat concentration ($p = 0.04$) were linearly increased, while milk urea nitrogen ($p < 0.01$) and somatic cell count ($p = 0.04$) were linearly decreased. Linear increases were also detected in the proportions of milk saturated fatty acids ($p < 0.01$) and polyunsaturated fatty acids ($p = 0.04$); however, milk monounsaturated fatty acids were linearly decreased ($p = 0.04$). The ruminal concentrations of acetate, propionate and butyrate increased, while the ruminal pH and the concentration of $\text{NH}_3\text{-N}$ reduced at a decreasing rate with increasing inulin doses ($p < 0.01$). Moreover, the concentrations of lactic acid ($p = 0.03$) and total volatile fatty acids ($p < 0.01$) were linearly upregulated. There were linear increases in the serum concentrations of superoxide dismutase ($p = 0.02$), immunoglobulin G ($p < 0.01$), immunoglobulin M ($p = 0.04$), interleukin-2 ($p < 0.01$) and interleukin-10 ($p = 0.04$); quadratic increases in serum total protein ($p < 0.01$) and albumin ($p = 0.02$) and linear decreases in serum total cholesterol ($p = 0.02$), triglyceride ($p < 0.01$) and malondialdehyde ($p < 0.01$). The results indicated that inulin increased milk production, shifted milk fatty acid profile, upregulated rumen volatile fatty acid concentration, and enhanced antioxidant and immunity function in dairy cows in a dose-dependent manner.

Keywords: dairy cows; inulin; milk fatty acids; serum metabolites; volatile fatty acids

1. Introduction

Inulin is a polydispersed fructan present in a wide range of plants, such as Chicory root (*Cichorium intybus* L.) [1] and Jerusalem artichoke (*Helianthus tuberosus* L.) [2]. Molecularly, inulin comprises several fructose units linked to each other with terminal glucose [3]. The degree of polymerization ranges between 2 and 60 in inulin with terminal fructose–glucose linked by a β -(1→2) glycosidic bond [4]. The β -oriented anomeric carbon of the fructose monomer is resistant to the hydrolysis of alimentary enzymes in the upper gastrointestinal tract, but quantitatively fermented in the colon of monogastric animals and humans [5].

Thus, inulin is often used as a prebiotic which is effective in selectively promoting the proliferation of probiotic bacteria such as *Bifidobacterium* and *Lactobacillus* and providing colonization resistance against invading pathogens, such as *E. coli* and *clostridia*, in the colon [6]. Various nutrition- and health-promoting functions of inulin have been reported including its ability to improve the intestinal environment, immunomodulation, glucose metabolism, and mineral absorption in humans [7]. There is also evidence that inulin can reduce inflammation by directly dampening the pathogen-induced activation of mitogen-activated protein kinase (MAPK) signaling in the intestinal mucosa, independent of gut microbes in mice [8].

On the other hand, the effects of inulin on lactating dairy cows are still not as quantitatively reported as those in monogastric animals. Rumen harbors a substantial and diverse population of symbiotic microorganisms. Therefore, inulin might be fermented and utilized by the ruminal microbes and host animals under a different mechanism compared to those in monogastric animals and humans. Hindrichsen et al. (2004) reported that the in vitro ruminal organic matter degradability was higher in the Jerusalem artichoke tuber diet (73.9%) than in the wheat diet (65.6%), with the two diets being iso-energetic and iso-nitrogenous [9]. Additionally, the organic matter and energy digestibility (74.5% and 71.6%) of the Jerusalem artichoke diet were also greater than those (70.3% and 66.5%) of the wheat diet, respectively, when offered to dairy cows in vivo [10]. Tian et al. (2019) reported that a 2% (wt/wt) dietary inulin addition increased the final bodyweight of finishing beef steers, with elevated ruminal concentration of propionate in the low concentrate diet and higher ruminal bacterial diversity in the high concentrate diet [11]. The effects of inulin on rumen fermentation and microbial community in vitro [12] and in calves [13] were also studied. However, the responses in milk yield, milk composition, milk fatty acid profile, rumen fermentation and serum biochemical parameters with different inulin addition levels in practically feeding lactating dairy cows still need further investigation. Furthermore, the effectiveness of inulin could be considerably impacted by the supplementation doses. We hypothesized that inulin could increase rumen volatile fatty acid concentrations, improve milk production, change milk composition and affect blood biochemical parameters with increasing doses. Therefore, the objective of the current study was to investigate the responses in milk yield, milk composition, milk fatty acid profile, rumen fermentation and blood antioxidant and immunity parameters with increasing dietary inulin doses in mid-lactation dairy cows.

2. Materials and Methods

2.1. Experimental Diets and Animals

The inulin (>85% purity, other sugars contained fructose, sucrose and glucose < 15%, 95% dry matter (DM) and 0.2% ash) was in powder form and extracted from Jerusalem artichoke tubers, supplied by the Langfang Academy of Agriculture and Forestry Sciences (Langfang, Hebei, China). The study was carried out in a dairy farm in Tongzhou District, Beijing, China. Thirty-six healthy mid-lactation Holstein dairy cows were blocked based on their average body weight (BW) (553 ± 17.3 kg), milk yield (33.7 ± 2.64 kg/d), days in milk (166 ± 48.8 d), parity (1.83 ± 0.91) and milk somatic cell counts (SCC) ($313 \pm 64.5 \times 10^3$ cells/mL) and then randomly assigned to 1 of 6 dietary groups, with 6 cows per group, balanced with all the above-mentioned factors. The cows were housed in a free-stall barn and offered the same basal total mixed ration (TMR) ad libitum with 0 (control), 50, 150, 200, 250 and 350 g/d inulin supplementation per cow, respectively. The ingredients and nutrient composition of the TMR are shown in Table 1. The cows were offered the same basal TMR three times a day at 0700 h, 1400 h and 1830 h, allowing for 5–10% refusals with free access to water. Due to hygroscopicity, the inulin was rolled into small balls with 50 g each and orally dosed to each individual cow according to their treatments during the morning feeding. The experiment lasted for 5 weeks with 2-week pre-feeding for adaption and 3 weeks for sampling.

Table 1. Ingredients and chemical composition of basal diet (% of DM, unless otherwise stated).

Ingredients		Chemical Composition	
Corn silage	44.20	Dry matter, % of fresh	46.00
Alfalfa hay, chopped	4.37	Crude protein	17.00
Oat grass	8.39	Neutral detergent fiber	29.40
Alfalfa leaf meal	2.17	Acid detergent fiber	16.60
Steam-flaked corn	5.22	Ether extract	4.22
Corn grain, ground	6.48	Ca	0.85
Soybean hull	2.17	P	0.52
Sugar beet pellets	2.17	Ash	8.10
Soya meal	8.74	Net energy for lactation, Mcal/kg DM	1.74
Cottonseed	3.48		
Corn bran	3.04		
Rapeseed meal	2.13		
Extruded soybean	1.74		
Megalac ¹	0.87		
Fatty powder ²	0.48		
5% Premix ³	4.35		

¹ Megalac, a rumen protected fatty acid calcium (VOLAC International Ltd., Royston, UK). ² Fatty powder contained $\geq 99.5\%$ ether extract and $\leq 0.5\%$ water and various impurities. Several specific fatty acid indicators (% of total Fatty acids), C16:0 ($\geq 60\%$), C18:0 ($\leq 38\%$), C18:1 ($\leq 5\%$), C18:2 ($\leq 5\%$), other fatty acids ($\leq 3\%$). ³ 5% Premix contained (per kg of DM) 400,000 IU of vitamin A, 320,000 IU of vitamin D3, 1200 IU of vitamin E, 1400 mg of Cu, 12,000 mg of Zn, 60,000 mg of Fe, 12,000 mg of Mn, 40 mg of Se, 400 mg of I, 160 mg of Co, 28% of Ca and 5.4% of P.

2.2. Feed Analysis

The TMR samples were collected on d 20, 21, 27, 28, 34 and 35 of the experiment and composited every two days to obtain 1 sample. The samples were dried in a forced air oven at 55 °C for 48 h and then ground passed through a 1 mm screen for the follow-up nutrient analysis. The TMR samples were analyzed for DM (105 °C, 4 h), ash (method 942.05; AOAC, 1990), crude protein (CP) (method 988.05; AOAC, 1990), ether extract (method 920.39; AOAC 1990), Ca (method 985.35; AOAC, 1990) and P (method 986.24; AOAC, 1990) [14]. The neutral detergent fiber and acid detergent fiber were determined according to Van Soest et al. (1991) using an ANKOM 220 fiber analyzer (ANKOM Technology, New York, NY, USA) with α -amylase and sodium sulfite and expressed inclusive of residual ash [15]. The concentration of net energy of lactation (NEL) was calculated according to NRC (NRC, 2001).

2.3. Rumen Fluid Sampling and Analysis

Rumen fluid samples were collected from each cow around 2 h after the morning feeding by an esophageal gavage on d 21, 28 and 35 of the experiment. The first 200 mL of rumen fluid was discarded to minimize saliva contamination, and then approximately 250 mL of rumen fluid was collected from each cow. The pH value of each rumen fluid sample was immediately determined by a portable pH meter (Omega Engineering Inc., Norwalk, CT, USA). After filtering through 4 layers of cheesecloth, each rumen fluid was divided into 3 portions for volatile fatty acids (VFAs), ammonia-nitrogen ($\text{NH}_3\text{-N}$), and lactic acid (LA) analyses, respectively. The concentrations of VFAs were determined by gas chromatography (Agilent 7890A, Agilent Technologies, Inc., Santa Clara, CA, USA), as described in our previous report [16]. The concentrations of $\text{NH}_3\text{-N}$ and LA were determined by the phenol-hypochlorite method using a microplate reader (Multiskan MK3, Thermo Labsystems, Philadelphia, PA, USA) and an LA colorimetric assay kit (BC2235, Solarbio, Beijing, China), respectively.

2.4. Milk Sampling and Analysis

The cows were milked 3 times a day at 0630 h, 1330 h and 2030 h using an Afimilk milking system (Side-by-Side Parallel Stall Construction, Afimilk Ltd., Kibbutz Afikim,

Israel). Milk yield of each cow per day was automatically recorded during the 3 sampling weeks. The BW of each cow was measured on the last day of each week before the morning milking. A 50 mL of milk sample was collected during each milking from each cow on d 21, 28 and 35 of the experiment. The milk samples collected in the morning, afternoon and evening were then mixed in a ratio of 4:3:3 into two 50 mL sterile centrifuge tubes with potassium dichromate (0.6 mg/mL) as a preservative and stored at 4 °C. One tube was used for milk composition analysis. Milk fat, protein, lactose, somatic cell count (SCC) and milk urea nitrogen (MUN) contents were analyzed by the CombiFoss™ 7 (Foss Electric A/S, Hillerød, Denmark). The other tube was used to analyze milk fatty acids (FAs), which were freeze-dried to a moisture content < 5%, and then subjected to methyl esterification extraction. The milk FAs were converted to fatty acid methyl esters (FAMES) using the acetyl chloride-methanol methyl esterification method as described by Jia et al. (2017) [17]. In brief, approximately 500 mg of sample was dissolved in 5 mL of toluene in a 20 mL screw capped tube. Derivatization was performed with 6 mL of 10% acetyl chloride-methanol solution at 80 °C for 2 h in a water bath with a shake every 20 min. After cooling to room temperature, the solution was quantitatively transferred to a 50 mL centrifuge tube. The screw capped tube was then washed three times using 3 mL of 6% sodium carbonate solution, which was also added into the 50 mL centrifuge tube. The sample was centrifuged at $2750 \times g$ for 5 min to harvest the supernatant, which was subsequently analyzed using an Agilent 7890A gas chromatograph system (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a flame ionization detector and a HP-88 chromatographic column (100 m $\times$ 0.25 mm $\times$ 0.20 μ m, Agilent Technologies, Inc., Santa Clara, CA, USA). The standard solution used was the 37-component reference standard mixtures of C4-C24 FAMES (2–4% relative concentration) (Supelco 18919, Supelco Co., Ltd., Shanghai, China). The standard solution was diluted in 10 mL hexane before use. The standard solution (1.0 μ L) and milk FAMES sample (1.0 μ L) were pipetted into sample vials for chromatographic analysis. The carrier gas was N₂ with a flow rate at 1.0 mL/min. The inlet and detector temperatures were 260 °C and 280 °C, respectively. The injection volume was 1 μ L with a split ratio of 30:1. The initial oven temperature was 140 °C for 5 min, and then increased to 240 °C at 4 °C/min and held for 15 min [18].

2.5. Blood Sampling and Analysis

Two 5 mL coagulation-promoting tubes (Corning, Corning Incorporated Costar, NY, USA) with glass particles and inactive silica as coagulant were used to collect blood samples from each cow on d 21, 28 and 35 of the experiment. Each tube harvested 3–4 mL of blood samples by tail venepuncture at the morning feeding. The samples were transferred to a laboratory immediately, centrifuged at $3000 \times g$ for 10 min to harvest serum and then stored at –20 °C for biochemical analyses. The serum triglyceride (TG) and total cholesterol (TC) concentrations were analyzed using a serum TG kit (TR0100, Sigma-Aldrich, St. Louis, MO, USA) and TC kit (MAK043, Sigma-Aldrich, MO, USA), respectively. The serum concentrations of total protein (TP) and albumin (ALB) were analyzed using a TP kit (A045-3, DiaSys Diagnostics Systems GmbH, Frankfurt, Germany) and an ALB kit (MAK124, Sigma-Aldrich, MO, USA), respectively. The concentration of serum globulin (GLO) was then calculated as the difference between the concentrations of TP and ALB. The serum concentrations of immunoglobulin A (IgA), IgG and IgM were analyzed using an automatic biochemical analyzer (Roche, Basel, Switzerland). The concentrations of interleukin (IL)-2 (H003), IL-6 (H007-1-1), IL-10 (H009-1), superoxide dismutase (SOD) (A001-3-2), malondialdehyde (MDA) (A003-1-2), glutathione peroxidase (GSH-Px) (A005-1-2), tumor necrosis factor (TNF)- α (H052-1), and IFN- γ (H025) were analyzed using commercial assay Kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

2.6. Statistical Analyses

Data of lactation performance, milk fatty acid proportions, rumen fermentation and serum biochemical parameters were analyzed using the repeated measures ANOVA in the GLM of SPSS Statistics (Version 22, IBM, Chicago, Armonk, NY, USA). The effect of week was included as a repeated measure. Week was set as the within-subject factor, inulin dose was set as the between-subject factor, and cow within a diet was set as a random effect. Linear and quadratic orthogonal polynomial contrasts were used to examine the responses of lactation performance, milk fatty acid proportions, rumen fermentation and serum biochemical parameters to different doses of inulin, with coefficients adjusted for unequal spacing in the inulin supplementation levels. The results were presented as least squares means. Significance was declared at $p < 0.05$, and a tendency was declared at $0.05 \leq p < 0.10$.

3. Results

3.1. Rumen Fermentation

Inulin decreased the ruminal pH and the concentration of $\text{NH}_3\text{-N}$, while on the other hand it elevated the concentrations of acetate, propionate and butyrate at a decreasing rate with increasing doses ($p < 0.01$, Table 2). However, the acetate to propionate ratio was not significantly changed. The concentrations of LA ($p = 0.03$) and total volatile fatty acids (TVFA) ($p < 0.01$) were linearly upregulated as inulin doses increased, while the concentrations of isobutyrate ($p = 0.04$) and valerate ($p = 0.06$) had and tended to have quadratic responses with their highest values at 350 and 250 g/d, respectively.

Table 2. Effects of inulin on rumen fermentation in dairy cows.

Items	Inulin, g/d per Head ($n = 6$)						SEM	p -Value	
	0	50	150	200	250	350		Linear	Quadratic
pH	6.56	6.48	6.39	6.30	6.34	6.34	0.015	<0.01	<0.01
Ammonia nitrogen, mg/dL	13.3	12.9	11.0	8.90	9.07	8.57	0.392	<0.01	<0.01
Lactic acid, mmol/L	0.84	0.87	0.90	0.92	0.89	0.91	0.008	0.03	0.26
Acetate, mmol/L	67.9	66.9	76.5	87.5	80.4	83.9	1.45	<0.01	<0.01
Propionate, mmol/L	25.2	23.5	28.5	30.3	29.6	31.2	0.59	<0.01	<0.01
Butyrate, mmol/L	11.0	10.3	12.3	13.6	14.2	14.2	0.40	<0.01	<0.01
Isobutyrate, mmol/L	1.42	1.49	1.63	1.69	1.67	1.72	0.070	0.30	0.04
Valerate, mmol/L	1.31	1.47	1.44	1.67	1.79	1.78	0.089	0.46	0.06
Isovalerate, mmol/L	1.94	2.12	2.54	2.65	2.77	2.68	0.062	0.62	0.47
Total volatile fatty acids, mmol/L	109	106	123	137	130	135	2.4	<0.01	0.08
Acetate/propionate	2.69	2.85	2.68	2.89	2.72	2.69	0.027	0.84	0.49

3.2. Animal Performance

The milk yield ($p < 0.01$), energy-corrected milk (ECM) ($p < 0.01$), fat-corrected milk (FCM) ($p < 0.01$) and milk fat rate ($p = 0.04$) linearly increased, and milk protein rate ($p = 0.08$) tended to linearly increase with the increasing inulin addition (Table 3), while milk lactose concentration increased quadratically with its highest value achieved at 250 g/d ($p < 0.01$). In contrast, inulin linearly downregulated the MUN ($p < 0.01$) and SCC ($p = 0.04$) contents. The BW of cows and milk fat-to-protein ratio were not affected by the inulin doses.

Table 3. Effects of inulin on animal performance and lactation in dairy cows.

Items ¹	Inulin, g/d per Head (n = 6)						SEM	p-Value	
	0	50	150	200	250	350		Linear	Quadratic
Body weight, kg	551	550	557	557	557	558	1.5	0.29	0.72
Milk yield, kg/d	33.5	34.1	34.6	36.0	35.3	37.0	0.17	<0.01	0.24
Energy-corrected milk, kg/d	32.4	33.1	33.9	37.1	34.9	36.8	0.33	<0.01	0.06
Fat-corrected milk, kg/d	32.3	33.1	33.9	36.8	34.8	36.4	0.35	<0.01	0.07
Milk fat, %	3.78	3.80	3.86	4.14	3.91	3.88	0.159	0.04	0.27
Milk protein, %	3.31	3.31	3.36	3.54	3.40	3.51	0.080	0.08	0.97
Milk fat/milk protein	1.14	1.15	1.15	1.17	1.15	1.11	0.013	0.96	0.25
Lactose, %	5.17	5.19	5.17	5.18	5.22	5.21	0.030	0.32	<0.01
Milk urea nitrogen, mmol/L	6.13	6.02	5.00	4.58	5.57	4.76	0.297	<0.01	0.48
Somatic cell counts, ×10 ³ /mL	314	316	311	278	316	259	4.9	0.04	0.17

¹ Energy-corrected milk (kg/d) = milk yield (kg/d) × (383 × fat (%) + 242 × protein (%) + 783.2)/3140; Fat-corrected milk (kg/d) = 0.4 × milk yield (kg/d) + 15 × milk yield (kg/d) × fat (%).

3.3. Milk Fatty Acids

With increasing inulin doses, the proportion of saturated FA (SFA) increased at a decreasing rate ($p < 0.01$), while the proportion of unsaturated FA (UFA) decreased quadratically ($p = 0.03$, Table 4). Within the UFA, the monounsaturated FA (MUFA) decreased linearly ($p = 0.04$), however the polyunsaturated FA (PUFA) increased linearly ($p = 0.04$). Specifically, there were linear increases in several individual SFA including C4:0 ($p < 0.01$), C8:0 ($p = 0.04$), C10:0 ($p = 0.03$), C13:0 ($p = 0.01$) and C16:0 ($p = 0.03$). In contrast, the proportion of C18:0 ($p = 0.04$) was linearly decreased, while for individual MUFA, the proportion of C14:1 ($p = 0.02$) decreased linearly and C18:1 *trans*-9 and C20:1 reduced at a decreasing rate ($p < 0.05$). On the other hand, in individual PUFA, the proportions of C18:2n6 *cis* and C18:3n6 increased at a decreasing rate with increasing inulin doses ($p < 0.01$). There were quadratic responses in the proportions of C20:3 *cis*-8,11,14 and C20:4 and they were both greatest at the dose of 200 g/d with the increasing inulin supplementation ($p < 0.01$).

Table 4. Effects of inulin on milk fatty acids in dairy cows.

Items, g/100 g FA	Inulin, g/d per Head (n = 6)						SEM	p-Value	
	0	50	150	200	250	350		Linear	Quadratic
Saturated fatty acids	68.6	70.4	72.7	76.0	71.9	75.2	0.65	<0.01	<0.01
C4:0	2.99	3.02	3.28	3.42	3.38	3.29	0.039	0.01	0.29
C6:0	2.14	2.28	2.20	2.25	2.46	2.22	0.050	0.47	0.72
C8:0	1.05	1.35	1.28	1.36	1.42	1.35	0.033	0.04	0.22
C10:0	2.40	3.05	3.12	3.38	2.77	3.35	0.091	0.03	0.11
C11:0	0.04	0.06	0.04	0.05	0.06	0.05	0.009	0.68	0.63
C12:0	3.38	3.56	3.60	3.60	3.63	3.57	0.129	0.83	0.07
C13:0	0.10	0.14	0.11	0.12	0.15	0.12	0.005	0.01	0.14
C14:0	11.1	12.2	11.3	11.8	11.5	11.6	0.22	0.87	0.06
C15:0	1.09	1.11	1.06	1.10	1.14	1.12	0.019	0.78	0.07
C16:0	30.3	29.3	31.0	33.5	31.2	33.1	0.39	0.03	0.14
C17:0	0.47	0.45	0.49	0.46	0.46	0.48	0.006	0.29	0.28
C18:0	9.86	9.53	9.61	8.18	8.38	8.57	0.267	0.04	0.13
C20:0	0.06	0.06	0.08	0.08	0.06	0.07	0.007	0.16	0.48
C21:0	0.16	0.11	0.15	0.14	0.25	0.17	0.026	0.10	0.62
C22:0	0.11	0.13	0.09	0.14	0.06	0.13	0.008	0.27	0.54
Unsaturated fatty acids	30.4	30.2	27.5	27.6	27.8	28.0	0.45	0.16	0.03
Monounsaturated fatty acids	27.6	27.3	24.1	23.9	24.3	24.5	0.42	0.04	0.18
C14:1	1.92	1.95	1.55	1.57	1.70	1.59	0.06	0.02	0.32

Table 4. Cont.

Items, g/100 g FA	Inulin, g/d per Head (n = 6)						SEM	p-Value	
	0	50	150	200	250	350		Linear	Quadratic
C15:1	0.33	0.30	0.27	0.27	0.32	0.29	0.009	0.26	0.41
C16:1	1.81	1.68	1.50	1.61	1.84	1.65	0.053	0.31	0.28
C17:1	0.20	0.15	0.14	0.15	0.13	0.15	0.007	0.68	0.13
C18:1 <i>trans</i> -9	0.58	0.51	0.54	0.43	0.48	0.51	0.013	0.03	0.04
C18:1 <i>cis</i> -9	20.2	20.1	17.6	17.3	17.3	17.7	0.40	0.25	0.86
C20:1	0.44	0.43	0.34	0.31	0.35	0.34	0.018	0.03	<0.01
C22:1	0.16	0.16	0.15	0.19	0.19	0.19	0.006	0.98	0.54
Polyunsaturated fatty acids	2.79	2.80	3.14	3.41	3.18	3.27	0.077	0.04	0.07
C18:2n6 <i>trans</i>	0.21	0.19	0.19	0.18	0.17	0.20	0.018	0.07	0.14
C18:2n6 <i>cis</i>	2.11	2.15	2.42	2.61	2.46	2.52	0.048	<0.01	<0.01
C18:3n6	0.10	0.11	0.20	0.23	0.17	0.20	0.026	<0.01	<0.01
C18:3n3	0.23	0.20	0.15	0.19	0.20	0.17	0.016	0.27	0.23
C20:2 <i>cis</i> -11,14	0.04	0.04	0.07	0.08	0.07	0.08	0.006	0.25	0.36
C20:3 <i>cis</i> -8,11,14	0.05	0.05	0.07	0.13	0.10	0.02	0.010	0.24	<0.01
C20:4	0.10	0.11	0.11	0.12	0.11	0.10	0.003	0.15	<0.01

3.4. Serum Metabolites

The serum TC ($p = 0.02$), TG ($p < 0.01$) and MDA ($p < 0.01$) concentrations were linearly downregulated as inulin doses increased (Table 5). Conversely, the concentration of SOD increased at a decreasing rate ($p < 0.05$) and GSH-Px tended to increase ($p = 0.07$) with inulin addition. Quadratic responses were detected in the concentrations of TP ($p < 0.01$) and ALB ($p = 0.02$) with their greatest values both obtained at 200 g/d. The IgG ($p < 0.01$) and IgM ($p = 0.04$) concentrations increased linearly with the increasing inulin doses. Meanwhile, the concentration of IL-2 increased linearly ($p < 0.01$) and that of IL-10 increased at a decreasing rate ($p < 0.05$) with increasing inulin supplementation.

Table 5. Effects of inulin on serum metabolites in dairy cows.

Items	Inulin, g/d per Head (n = 6)						SEM	p-Value	
	0	50	150	200	250	350		Linear	Quadratic
Total cholesterol, mmol/L	7.88	7.64	8.42	6.67	7.50	7.18	0.159	0.02	0.06
Triglyceride, mmol/L	0.47	0.45	0.46	0.41	0.44	0.43	0.004	<0.01	0.15
Malondialdehyde, nmol/L	2.94	2.89	2.68	2.02	2.14	2.25	0.085	<0.01	0.21
Superoxide dismutase, U/mL	48.1	48.9	51.5	52.5	51.7	50.1	0.28	0.02	<0.01
Glutathione peroxidase, μ mol/L	7.84	7.87	7.90	8.27	8.30	8.06	0.081	0.07	0.49
Total protein, g/L	70.8	71.1	74.9	75.9	75.1	71.0	0.53	0.70	<0.01
Albumin, g/L	34.9	34.1	34.3	36.3	35.8	34.0	0.30	0.57	0.02
Globulin, g/L	35.9	37.0	40.6	39.6	39.3	37.0	0.60	0.97	0.26
Immunoglobulin A, mg/mL	0.14	0.12	0.16	0.16	0.14	0.17	0.002	0.77	0.19
Immunoglobulin G, mg/mL	3.25	3.11	2.89	4.29	2.77	4.12	0.087	<0.01	0.29
Immunoglobulin M, mg/mL	1.06	1.12	0.91	1.25	1.22	1.21	0.030	0.04	0.19
Interleukin-2, pg/mL	193	196	175	231	171	244	4.4	<0.01	0.14
Interleukin-6, pg/mL	73.0	76.1	69.7	63.7	65.2	68.4	1.61	0.83	0.64
Interleukin-10, pg/mL	109	111	132	143	138	158	3.8	0.04	0.03
Tumor necrosis factor- α , pg/mL	247	253	268	284	255	277	7.5	0.31	0.68
Interferon- γ , pg/mL	62.4	63.7	65.0	65.1	66.7	62.9	1.95	0.70	0.35

4. Discussion

4.1. The Effects of Inulin on Rumen Fermentation

The pH in rumen was decreased linearly by inulin doses. The dropped pH was in line with the elevated TVFA concentration in the rumen, which might be attributed to the shifted rumen microbiota structure and consequently the improved concentrations

of individual VFAs (i.e., acetate, propionate and butyrate) induced by inulin. In our previous study [16], inulin increased the relative abundances of *Saccharofermentans* (acetate-producing) [19], *Muribaculaceae* (propionate-producing) [20], *Prevotellaceae_NK3B31_group* (acetate- and butyrate-producing) [21], *Treponema* (acetate- and butyrate-producing) [22], *Butyrivibrio* (butyrate-producing) [23], *Acetitomaculum* (acetate- and LA-producing) [24] and *Eubacterium_hallii_group* (propionate- and LA-producing) [25] in the rumen of dairy cows. The proliferation of these VFAs and LA-producing bacteria may explain the linearly increased ruminal concentrations of acetate, propionate, butyrate and LA. Additionally, the elevated concentrations of these individual VFAs consequently contributed to the increased TVFA in the current study. Meanwhile, the reduced concentration of $\text{NH}_3\text{-N}$ with the inulin supplementation may indicate enhanced N utilization efficiency in the microbial crude protein (MCP) synthesis through better energy and N synchronization, as more readily available energy is provided by the increased VFAs. Moreover, valerate and isobutyrate are growth factors for cellulolytic bacteria in the rumen [26] and are produced via the deamination and decarboxylation processes of the branched-chain amino acids [27]. The quadratic increase in their concentrations in the present study probably also indicated that inulin supplementation facilitated the growth of rumen cellulolytic bacteria, which promoted fiber digestion as that reported in beef cattle [11] and upregulated amino acid metabolism as described in our previous report [16].

4.2. The Effects of Inulin on Lactation Performance

Inulin supplementation linearly improved milk production of the mid-lactation cows in the current study. This could be attributed to the increased TVFA that provided extra energy and more substrates for milk synthesis. The sweetness of inulin may enhance the appetite of the dairy cows as well [5]. The increasing inulin doses also linearly increased milk fat concentration and tended to increase milk protein concentration. The elevated milk fat level could be a result of the increased ruminal acetate and butyrate concentrations, which acted as the main precursors for de novo milk FA synthesis [28], while the trend of increased milk protein content could be derived from the enhanced MCP synthesis through better energy and N synchronization. This is evidenced by the linearly increased TVFA and decreased ruminal $\text{NH}_3\text{-N}$ and MUN concentrations, as discussed previously. Hall and Weimer (2016) reported that the MCP yield could be 20% greater for inulin compared with glucose when fermented in vitro with ruminal microbes [29]. Moreover, de novo milk FA production also has a positive relationship with milk protein production [30]. The increase in milk fat and protein concentrations consequently resulted in the promoted yields of ECM and FCM. Inulin quadratically increased milk lactose concentration, which might be due to the elevated ruminal propionate concentration in the current study. Because propionate is quantitatively the greatest contributor to gluconeogenesis in the liver [31], and glucose is the precursor for the synthesis of lactose in the mammary gland [32]. Milk SCC was linearly reduced with increasing inulin doses. The decreased milk SCC practically reflected the reduction in inflammatory response in the udder [33] and indicated the possible effect of inulin in the enhancement of immune and disease resistance in dairy cows.

4.3. The Effects of Inulin on Milk Fatty Acids

Inulin supplementation linearly increased the proportions of SFA in milk. This could be attributed to the increased de novo FA synthesis. The SFA including C4:0 to C12:0, 95% of 14:0 and about half of C16:0 in milk are synthesized in the mammary gland by acetate and butyrate [28]. Similarly, our results have also seen the linear increase in acetate and butyrate concentrations in the rumen of cows in responding to the dietary supplementation of inulin. De novo fatty acid synthesis produces C16:0 as the final end product, while C18:0 is all derived from the small intestine absorption and adipose mobilization [28]. The long-chain FA inhibits de novo synthesis of short-chain FA through decreasing the activity of acetyl CoA carboxylase, which acts as the primary regulator for FA synthesis [34]. This may explain the downregulated C18:0 proportion as de novo FA synthesis was enhanced

in the current study. Moreover, the SFA proportion ranged from 68.6 to 76.0 g/100 g FA with the greatest contribution from C16:0 in the current study, which was similar to the results (SFA represented 60–70% of FA with C16:0 as the most abundant) in the study of Wang et al. (2022) [35]. In contrast, inulin linearly reduced the proportion of MUFA, indicating a negative correlation with SFA. The relative proportions of MUFA and SFA are closely related to energy balance. The increase in MUFA is usually derived from greater lipidic reserves mobilization during the net energy balance, such as in early lactation or feed restriction, whereas SFA would increase with increasing energy supply [36]. Specifically, the proportion of C18:1 *trans*-9 was linearly reduced with the increasing inulin doses in the current study. Kadegowda et al. (2008) observed a significant negative effect of C18:1 *trans*-9 on milk fat percentage [37]. Meanwhile, its reduction may also provide health benefits, as consuming a C18:1 *trans*-9-rich diet stimulated atherosclerosis in mice [38]. Moreover, C18:1 *cis*-9 was the most prominent MUFA (17.3–20.2 g/100 g FA) in the current study, which was within the range of 14–24% reported by Chilliard and Ferlay (2004) [39] and the sum of C18:1 and C18:0 are almost equal to the C16:0 content in normal milk fat [34]. Inulin doses linearly increased the proportions of PUFA including C18:2n6 *cis* and C18:3n6 and quadratically increased those of C20:3 *cis*-8,11,14 and C20:4 in the current study. This was possibly related to the decreased biohydrogenation activity in rumen and/or certain microorganisms involved in the biohydrogenation and/or increased mammary desaturation [28]. In addition, the proportion of C18:2n6 was predominant in the PUFA and within the typical range (2 and 3%) in milk FA [39]. A higher proportion of PUFA in milk fat is desirable from the perspective of human health against cardiovascular diseases, indicating the potential benefit of inulin in promoting milk FA profile [40]. However, the rumen microorganisms involved in the biohydrogenation affected by inulin and the associated mechanism still require further investigation.

4.4. The Effects of Inulin on Serum Metabolites

Inulin doses linearly reduced the concentrations of TC and TG in serum. The increased propionate concentration in the rumen in the current study can lead to increased glucose production as discussed previously, which, in turn, stimulates insulin production and suppresses lipolysis in adipocytes [41]. On the other hand, the expression and activity of liver lipogenesis can also possibly reduce. Beylot (2005) reported that the inhibition of liver lipogenesis could be due to the selective exposure of the liver to the increased production of propionate through the fermentation of inulin in the large intestine in rodents [42]. Furthermore, Han et al. (2013) have also shown that inulin can reduce the activity and expression of FA synthase in the liver, resulting in a decrease in lipogenesis and therefore exhibiting hypolipidemic effect in rats [43]. The downregulated TG also agreed with the decreased milk C18:0 FA in the current study, as they are almost solely derived from serum TG [34]. The decrease in MDA and increase in SOD and GSH-Px with inulin addition were also observed in the serum. Malondialdehyde is a lipid peroxidation product of cell membrane and its content can indirectly reflect a degree of cell damage. Superoxide dismutase and GSH-Px are key enzymes of the antioxidant system in scavenging free radicals, resisting oxidative damage and maintaining cell structure [44]. The results of the current study indicated that dietary inulin supplementation might enhance the ability to resist oxidative stress in dairy cows. Greater serum ALB concentrations have previously been detected in higher yielding cows [45], which is in line with our finding that the cows that offered 200 g/d inulin had both the greatest FCM and serum ALB concentrations. Changes in TP levels were due mostly to the variations in ALB concentration in the present study, as no significant response was detected in the concentration of GLO. The IgG and IgM concentrations in the serum both increased linearly in responses to inulin doses. Previous studies have already suggested that inulin could upregulate the levels of IgG and IgM in the serum of rats and weaned piglets [3,46]. Immunoglobulin G and IgM are antibodies involved in humoral immunity. Inulin with certain immunogenicity can act as a cofactor

of immune stimulation to activate the immune response, thereby enhancing humoral immunity [46,47]. The concentrations of IL-2 increased linearly, and IL-10 increased at a decreasing rate in serum with increasing inulin supplementation. Interleukin-2, mainly secreted by CD4⁺ T lymphocytes, is best known for promoting T cell proliferation and mediating cellular immunity [48], while IL-10 is broadly expressed by many immune cells of both the adaptive and innate immune systems, playing a crucial role in mediating host anti-inflammatory response [49]. Dietary inulin has proven to enhance functions of the immune systems in mice and humans, especially resulting in greater resistance to tumor development through activating immune cells with elevated concentrations of short-chain fatty acids [47,50]. The results of the current study indicated that inulin could possibly strengthen the immune function in lactating dairy cows, consequently leading to decreased milk SCC, as previously stated. However, its mechanism still needs further investigation.

5. Conclusions

This study revealed that increasing dietary supplementation of inulin in mid-lactation dairy cows linearly increased milk yield, milk fat content, ECM, FCM, and milk PUFA proportion, tended to linearly increase milk protein content and linearly reduced MUN, milk SCC and milk MUFA proportion. The proportion of milk SFA increased at a decreasing rate with increasing inulin doses, while milk lactose concentration increased quadratically and maximized at 250 g/d inulin. For rumen fermentation, increasing inulin doses also linearly increased the concentrations of LA and TVFA, and linearly decreased the ruminal pH and the concentration of NH₃-N. The concentrations of acetate, propionate and butyrate increased at a decreasing rate with increasing inulin doses, while the concentrations of isobutyrate and valerate had and tended to have quadratic responses and maximized at 350 and 250 g/d inulin, respectively. In addition, the antioxidant capacity and immunity function were enhanced with linearly reduced TC, TG and MDA and linearly elevated IgG, IgM and IL-2 concentrations in serum. The serum concentrations of SOD and IL-10 increased at a decreasing rate, while GSH-Px tended to linearly increase with increasing inulin doses. Moreover, quadratic increases were detected in the serum concentrations of TP and ALB, with their greatest values obtained at 200 g/d inulin. In conclusion, dietary supplementation of inulin effectively improved the lactation performance, rumen fermentation, and antioxidant and immunity function in dairy cows in a dose-dependent manner. However, the mechanisms of inulin in rumen biohydrogenation and immunoregulation still need further investigation.

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