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Research article

Effects of glyphosate contamination in dairy cow diet on feed digestibility, nitrogen balance, milk production and blood profiles of lactating dairy cows

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Abstract

Importance of the work: Glyphosate (GL) is one of the most widely used herbicides globally. However, GL residues in feed may affect milk production and composition in dairy cows, raising concerns about its potential impacts on dairy performance and animal health.

Objectives: To evaluate the effects of concentrate feed contaminated with low or high levels of GL on milk production, nutrient digestibility, nitrogen balance and blood profiles in lactating dairy cows.

Materials and Methods: Three crossbred Holstein Friesian cows, with mean values ± SD of 33.67±18.18 days in milk and 16.75±1.99 kg daily milk yield, were allocated to a 3 × 3 Latin square design. The experimental treatments consisted of a control group, a Low-GL group (4 mg/kg dry matter, DM), and a High-GL group (8 mg/kg DM).

Results: Feeding dairy cows with the Low-GL or High-GL diets did not affect feed intake, milk yield or milk composition. However, lactose production decreased when cows were exposed to 47.52 mg/d GL. The DM nitrogen-free extract content increased linearly with higher dietary glyphosate levels, as did fecal nitrogen excretion. There were no significant effects on the blood profiles based on complete blood counts; however, GL exposure exceeding 34.75 mg/d led to decreased proportions of neutrophils and eosinophils and an increased proportion of lymphocytes.

Main finding: Concentrate feed contaminated with GL at a daily exposure of 47.52 mg had no significant effects on milk production, nutrient digestibility, nitrogen balance or blood cell counts. However, the studied GL exposures decreased lactose production and altered the specific blood cell proportion, with reduced neutrophil and eosinophil counts and an increased lymphocyte proportion.

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Introduction

Glyphosate (GL) is the most commonly applied herbicide used to control weeds in Thailand's agricultural industry, with associated impacts on the ecosystem (Gandhi et al., 2021). Although some countries have restricted the use of GL, it makes up 92% of the total herbicide sold globally (Antier et al., 2020) owing to advertising, despite concern about its impacts on animal production and health. GL's mode of action inhibits the synthesis of aromatic amino acids in plants and microorganisms by inactivating 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, resulting in weed death (Bentley and Haslam, 1990). EPSP synthase is not detected in mammals because it has relatively low toxicity to mammalians (Baer and Marcel, 2014). Despite its restricted use, GL contamination can still be discovered in the environment (Silva et al., 2018), food (Zoller et al., 2018) and animal feed, such as dairy and beef cattle feed (von Soonten et al., 2016). GL contamination has been found at 100 ng/g concentrations in soybean seeds and 40 ng/g in corn seeds (Duke et al., 2018). Meeprom et al. (2024) indicated that dairy cow feed in Surin Province, Thailand contained GL (430 ng/g). The quantity of GL contamination in animal feed is determined by various parameters, including harvest time, application concentration, application method and national legislation (Bote et al., 2019). GL residues in soybeans in Thailand have been reported in the range 0.07–0.53 mg/kg (Foundation for Consumers, 2020). Crops, including soybeans, rapeseed and corn, have also been genetically modified to be GL-resistant and since 1996, the number of genetically modified plants has grown rapidly (Duke and Powles, 2008). The maximum daily dose for ruminants with minimal negative effects is 50 mg/kg body weight, according to the European Food Safety Association (EFSA, 2015). However, ruminant microbes play a role in feed utilization and cause detrimental effects on animal production. For example, Hüther et al. (2005) reported that GL interfered with rumen microorganisms, as well as having high susceptibility to bacteria-degraded fiber (Ackermann et al., 2015). Changes in rumen microbiota were associated with milk production and composition in dairy cows (Liu et al., 2016). Still, low levels of GL had no effects on milk production and milk composition (Schnabel et al., 2017). Schnabel et al. (2017) observed that GL intake at 79.1 mg/d did not affect milk production or composition. Furthermore, Donkin et al. (2003) concluded that the influence of GL did not affect the efficacy and composition or milk. It could be suggested that the milk is not the excretion route for GL, since it is excreted primarily in feces (accounting for an average of 61%) and through the kidneys or urine (accounting for an average of 8%), with the remaining 31% being destroyed by microbes in the rumen. Therefore, GL leaves no traceable residue in milk (Von Soosten et al., 2016). Nonetheless, it has been reported that varied dietary energy requirements can considerably impact the concentration of protein, fat, lactose and urea in milk, somatic cells, and the fat-to-protein ratio (Donkin et al., 2003). In addition, Heymann et al. (2023) reported significant effects from GL on white blood cells in calves by reducing the total leukocytes compared to the control group, as well as affecting the proportions of lymphocytes and granulocytes. Naz et al. (2019) found that when the GL level in rabbit feed increased, white blood cells increased, while hemoglobin and hematocrit decreased.

There have been a few studies on the effects of a high GL level in the diet on the milk production, nutrient digestibility, nitrogen balance and blood profile of dairy cows. Therefore, the current study investigated the effects of a crossbred Holstein-Frisian lactating dairy cow diet contaminated with low and high levels of GL on the feed intake, milk production, total tract digestibility, nitrogen balance and blood cell population.

Materials and Methods

Statement of animal rights

The study was conducted at the Department of Animal Science, Faculty of Agriculture and Technology, Rajamangala University of Technology Isan Surin Campus, Surin, Thailand. This study was approved by the Ethics Committee of the Institutional Animal Care and Use Committee at Rajamangala University of Technology Isan, Thailand (Approval no. 22/2565).

Treatment preparation

The contamination of GL in dairy feed was prepared using 480 mg/mL GL concentration (Shandong Weifang Rainbow Chemical Co., Ltd, PRC), diluted to 4 mg/mL and 8 mg/mL as GL-diluted solutions for mixing with the dairy cow concentrate feed. The concentrate feed was sampled to determine dry matter (DM) for calculating the contamination of GL herbicides at 4 mg/kg DM (Low-GL) and 8 mg/kg DM (High-GL) of concentrate feed. Before feeding the concentrate feed to the dairy cows, the GL-diluted solution was added to 50 mL water for spraying into the concentrate feed.

Animals and experimental design

Three crossbred Holstein Friesian lactating cows with mean values ± SD of 33.67±18.18 days in milk, 16.75±1.99 kg milk yield, 72±6 mth age and 360±17 kg initial weight were assigned to a 3 × 3 replicated Latin square design. The experiment consisted of three periods, with 21 d for each period, broken down into 7 d for adaptation and 14 d for sample collection. The 3 × 3 replicated Latin square design helped to address variability and to ensure the validity of the results by using a similar experimental unit (no. of lactations, days in milk, milk yield and breed type) which help to reduce animal variation. Furthermore, rumen ecology from individual cows served as an effective experimental unit to detect treatment effects. The treatments consisted of: (1) a control; (2) 4 mg/kg DM (Low-GL); and (3) 8 mg/kg DM (High-GL). The 4 mg/kg DM was designed to represent moderate contamination resulting from being fed normally (grains, silage, forage) under non-herbicide management and for 8 mg/kg DM as a high level of contamination that could occur in heavily sprayed crops or improperly handled feed. The feed offered was divided into three meals of 3 kg at 0600 hours, 2 kg at 1100 hours and 3 kg at 1600 hours. The concentrate feeds were provided along with Napier silage (*Pennisetum purpureum* Pakchong 1) at five meals per day (0700 hours, 1000 hours, 1300 hours, 1700 hours and 2100 hours) *ad libitum.* The feed chemical compositions are presented in Table 1. The three dairy cows were housed in individual pens, with each having *ad libitum* access to clean water and mineral blocks.

Feed intake and chemical analysis

During the collection period, the cows were weighed to calculate the feed ingestion-to-body weight ratio. The daily feed intake was recorded and the feed offered and refused by each cow were collected and sampled to determine their DM content using a hot-air oven at 60°C for 72 hr for each cow and using the constant weight to calculate the DM intake. The feed samples after drying were ground through a 1 mm screen for proximate analysis, according to the standard method of Association of Official Analytical Chemists (1998). The GL content was analyzed using the procedure from Tsuji et al. (1997).

Milk production and composition

Each dairy cow was milked twice daily at 0500 hours and 1600 hours. The milk production was recorded for individual cows using a hanging weight scale. On the last 4 d of each period, the milk samples were collected in the evening and morning for four consecutive days and stored at 4°C to evaluate the milk composition content using a Foss MilkoScan™ 7RM instrument (Denmark), with the somatic cell count determined using a Fossomatic™ 7DC instrument (Denmark).

Total tract digestibility and nitrogen balance

The total tract digestibility was measured for two consecutive days during days 19–21 of each period using the total collection method. Before data collection began, the three milking cows were acclimated for 3 d in a stanchion barn. The collection was carried each period from 0700 hours on the first collection day until 0700 hours the next day, on all 3 d. Fecal and urine collection was monitored 24 hr daily to ensure all feces and urine were accounted for. The feces were collected using a shovel after each defecation. To inactivate the bacteria, the droppings were placed in prepared receptacles, each uniquely identified and treated with 10 M H_2SO_4. The urine was collected using a clean container each time a cow urinated. Teach urine sample was collected and mixed with 10 M H_2SO_4, as for the fecal collection procedure. After collection the separate weights of the feces

Table 1 Feed chemical compositions used

Item	Treatment			Napier grass silage
	Control	Low-GL	High-GL	
Dry matter (%)	88.88	88.65	88.67	24.73
Glyphosate (mg/kg DM)	0.59	4.90	6.70	0.00
		% DM		
Crude protein	18.49	20.02	20.84	9.03
Crude fiber	5.48	5.64	5.80	33.03
Ether extract	0.87	0.72	0.73	2.14
Nitrogen-free extract	60.76	60.26	58.27	46.35
Ash	14.40	13.36	14.36	9.45

DM = dry matter; GL = glyphosate; Low-GL = 4 mg GL/kg DM; High-GL = 8 mg GL/kg DM

and urine were recorded. The total feces were collected and placed in a refrigerator daily from the morning until the next morning, when they were pooled and then weighted at the end of each day before sampling 10% for proximate analysis, according to Association of Official Analytical Chemists (1998). The urine was collected and acidified with 10M H_2SO_4 to prevent any nitrogen loss. Milk samples were collected to analyze milk nitrogen. Nutrients from the concentrate and Napier grass silage intake were determined by recording the daily weights of the offered and refused components. Samples of all the feed offered and refused were analyzed using a similar method to the proximate analysis of the feces. The total tract digestibility was calculated based the nutrients in feces-to-nutrient intake ratio. The nitrogen balance was calculated based on National Research Council (1989) according to Equation 1:

$$\text{Nitrogen balance} = \text{Nitrogen intake} - (\text{Fecal nitrogen} + \text{Urinary nitrogen} + \text{Milk nitrogen}) \quad (1)$$

Blood profile measurement

Cattle blood was collected after milking and before the morning feeding during the 21 d of each experimental period. A blood sample (each 3 mL) was collected from the tail vein (coccygeal vein) on the ventral surface of the tail of each animal into an ethylenediaminetetraacetic acid-coated vacutainer tube and placed in an ice box before transferring directly to the laboratory. Hematological data were determined from each blood sample using a fully automated hematology analyzer (Mythic 18 vet; Orphée S.A.; Switzerland.)

Statistical Analysis

All data were analyzed using a 3 × 3 replicated Latin square design with the Proc GLM SAS on Demand for Academics (SAS Institute Inc.; United States of America). Treatment means were compared using the least significant difference test. Differences among means were considered significant at $p < 0.05$. Trend analysis was performed using orthogonal polynomial contrasts.

Results

Feed intake

The dairy cows receiving either the Low-GL or High-GL concentrate feed did not have a significantly different intake of Napier grass silage compared to the control group, as shown in Table 2.

Table 2 Effects of feed-contaminated glyphosate on dry matter intake and glyphosate intake

Item	Treatment			SEM	*p* Value	
	Control	Low-GL	High-GL		Trt	L
Grass silage intake						
kg/d	8.07±1.36	7.95±1.50	7.90±1.04	0.351	0.942	0.764
%BW	2.26±0.38	2.22±0.44	2.22±0.38	0.092	0.931	0.770
$\%BW^{0.75}$	1.84±0.23	1.81±0.27	1.82±0.23	0.057	0.940	0.802
Concentrate intake						
kg/d	7.11±0.10	7.09±0.11	7.09±0.11	0.005	0.200	0.134
%BW	2.00±0.14	1.98±0.08	1.99±0.15	0.006	0.406	0.415
$\%BW^{0.75}$	1.68±0.09	1.67±0.05	1.67±0.09	0.005	0.500	0.452
Total dry matter intake						
kg/d	15.18±1.43	15.04±1.50	14.99±0.99	0.354	0.933	0.749
%BW	4.26±0.45	4.20±0.47	4.21±0.48	0.091	0.896	0.735
$\%BW^{0.75}$	3.52±0.23	3.48±0.25	3.49±0.25	0.049	0.880	0.712
Glyphosate intake						
mg/d	$4.19±0.06^{c}$	$34.75±0.56^{b}$	$47.52±0.73^{a}$	0.223	<0.001	<0.001
µg/BW	$11.70±0.76^{c}$	$97.09±3.72^{b}$	$133.29±9.88^{a}$	2.721	<0.001	<0.001
$µg/BW^{0.75}$	$50.92±2.60^{c}$	$422.24±10.47^{b}$	$579.06±33.91^{a}$	9.505	<0.001	<0.001

Values (mean ± SD) within the same row, superscripted with different lowercase letters, differ significantly ($p < 0.05$). SEM = standard error of the mean. DM = dry matter; GL = glyphosate; Low-GL = 4 mg GL/kg DM; High-GL = 8 mg GL/kg DM); BW = body weight; Trt = treatment; L= linear.

Milk production and composition

There was no significant effect of the concentrate feed-contaminated GL on milk production, milk composition or somatic cell count (Table 3). However, milk yield, lactose production and SNF decreased significantly in a linear fashion when more GL was added to the diet, as well as lactose production that resulted in decreased solid-not-fat DM

Total tract digestibility

The dairy cows that received the concentrate feed contaminated with GL had a significant linear decrease in DM and nitrogen-free extract digestibility when there was more GL in the diet (Table 4). There were no significant differences for organic matter, crude protein, crude fiber and nitrogen-free extract from receiving the concentrate contaminated with GL herbicide. However, there was a highly significant ($p < 0.10$) linear decrease in organic matter and crude protein digestibility.

Table 3 Effects of feed-contaminated glyphosate on milk production and composition

Item	Treatment			SEM	*p* Value	
	Control	Low-GL	High-GL		Trt	L
Milk yield (kg/d)	13.27±1.26	13.18±1.44	12.77±2.79	0.081	0.086	0.049
4% FCM (kg/d)	12.33±0.86	12.20±1.41	12.05±1.92	0.098	0.339	0.187
ECM (kg/d)	13.20±0.79	13.01±1.39	12.84±2.07	0.047	0.303	0.165
Fat						
(%)	3.57±0.67	3.51±0.37	3.68±0.44	0.058	0.308	0.296
(g/d)	469±59	462±65	463±60	7.037	0.795	0.640
Protein						
(%)	2.76±0.24	2.70±0.12	2.75±0.23	0.045	0.660	0.821
(g/d)	365±7	356±41	348±60	5.784	0.329	0.181
Lactose						
(%)	4.82±0.12	4.85±0.14	4.80±0.21	0.026	0.525	0.506
(g/d)	641±71[a]	640±84[a]	615±156[b]	1.993	0.019	0.012
Solids-not-fat						
(%)	8.19±0.17	8.13±0.13	8.11±0.34	0.052	0.605	0.392
(g/d)	1,086±91[a]	1,073±134[a]	1,038±238[b]	5.669	0.049	0.026
Total solids						
(%)	11.72±0.89	11.60±0.34	11.83±0.63	0.097	0.415	0.506
(g/d)	1,548±101	1,529±177	1,504±286	10.387	0.178	0.093
SCC (×10^{3}cells/ml)	55.25±41.21	76.32±88.91	92.33±48.54	34.782	0.776	0.529

Values (mean ± SD) within the same row, superscripted with different lowercase letters, differ significantly ($p < 0.05$). SEM = standard error of the mean. DM = dry matter; GL = glyphosate; Low-GL = 4 mg GL/kg DM; High-GL = 8 mg GL/kg DM); FCM = fat corrected milk; ECM = energy corrected milk; SCC = somatic cell count; Trt = treatment; L= linear.

Table 4 Effects of feed-contaminated glyphosate on total tract digestibility

Item (%)	Treatment			SEM	*p* Value	
	Control	Low-GL	High-GL		Trt	L
Dry matter	60.97±1.18	57.86±4.23	52.64±0.56	1.190	0.074	0.038
Organic matter	65.46±0.64	62.60±2.89	58.19±1.86	1.458	0.136	0.071
Crude protein	68.73±2.00	70.90±5.19	64.35±3.32	0.865	0.065	0.072
Crude fiber	42.70±1.82	34.77±7.08	25.60±4.82	4.290	0.200	0.106
Nitrogen-free extract	69.94±0.96	65.86±3.03	63.58±3.54	0.923	0.075	0.039
Ether extract	62.73±2.72	62.05±2.40	61.83±2.30	0.373	0.386	0.322

Values presented as mean ± SD; SEM = standard error of the mean.
DM = dry matter; GL = glyphosate; Low-GL = 4 mg GL/kg DM; High-GL = 8 mg GL/kg DM); Trt = treatment; L= linear.

Nitrogen balance

The nitrogen balance from the cows receiving a concentrate diet contaminated with 34.75 mg/d GL and 47.52 mg/d GL did not significantly affect the nitrogen balance (Table 5). However, the dairy cows receiving the High-GL concentrate feed tended to exhibit increased fecal nitrogen excretion.

Blood profiles

There were no significant effects on the red blood and white blood cell complete blood count in the cows fed with GL in the concentrate diet (Table 6). However, the white blood cell differential count differed in neutrophil percentage, lymphocyte percentage and eosinophil percentage. The cows consumed a diet containing GL had a decrease in neutrophils, while lymphocytes significantly increased (Fig. 1). The Low-GL and High-GL cows had lower eosinophil percentages than the control group. However, the percentages of monocytes and basophils were not significantly different among treatments.

Table 5 Effects of feed-contaminated glyphosate on nitrogen balance

Item (g/d)	Treatment			SEM	*p* Value	
	Control	Low-GL	High-GL		Trt	L
Nitrogen intake	264.81±12.21	281.49±7.31	288.22±8.87	4.656	0.129	0.070
Feces nitrogen	82.80±5.12	81.91±9.94	102.75±14.23	2.884	0.056	0.039
Urinary nitrogen	115.61±53.65	71.06±57.48	80.31±28.54	27.319	0.574	0.457
Milk nitrogen	50.00±1.11	47.43±0.75	47.55±2.71	3.124	0.830	0.643
Nitrogen balance	16.39±42.57	57.61±52.68	81.09±48.88	30.741	0.468	0.443

Values presented as mean ± SD; SEM = standard error of the mean.
DM = dry matter; GL = glyphosate; Low-GL = 4 mg GL/kg DM; High-GL = 8 mg GL/kg DM); Trt = treatment; L= linear.

Table 6 Effects of feed-contaminated glyphosate on complete blood count

Item	Treatment			SEM	*p* Value	
	Control	Low-GL	High-GL		Trt	L
Red blood cells ($\times10^6$/µL)	4.09±0.07	4.12±0.19	4.31±0.43	0.115	0.500	0.322
Hemoglobin (g/dL)	6.47±0.49	7.03±0.32	6.93±1.05	0.126	0.148	0.120
Hematocrit (%)	21.43±1.00	22.87±1.31	22.73±3.75	0.535	0.314	0.228
Mean corpuscular volume (fL)	52.33±3.13	55.40±1.05	52.70±6.09	1.776	0.529	0.897
Mean corpuscular hemoglobin (pg)	15.83±1.33	17.06±0.15	16.10±1.59	0.491	0.364	0.738
MCHC (g/dL)	30.17±1.05	30.76±0.80	30.53±0.59	0.189	0.282	0.304
Red blood cell distribution width (%)	21.20±1.87	21.00±1.42	21.60±1.21	0.360	0.582	0.514
Platelet count ($\times10^4$/µL)	17.70±19.26	28.83±12.48	24.53±10.33	3.7924	0.313	0.330
Mean platelet volume (fL)	5.83±0.78	6.43±0.74	6.30±0.70	0.309	0.491	0.398
Platelet distribution width (%)	10.90±0.64	9.67±2.76	11.07±1.56	0.365	0.275	0.777
Plateletcrit (%)	0.089±0.18	0.098±0.09	0.132±0.02	0.047	0.814	0.587
White blood cell ($\times10^3$/µL)	6.50±0.95	9.73±2.46	7.23±0.98	0.985	0.252	0.651
Lymphocytes ($\times10^3$/µL)	1.90±1.65	3.73±4.28	2.47±2.61	1.050	0.556	0.739
Lymphocytes (%)	45.00±6.12	55.97±16.20	47.30±16.91	11.042	0.784	0.896
Monocytes ($\times10^3$/µL)	0.27±0.25	0.33±0.31	0.33±0.29	0.050	0.636	0.452
Monocytes (%)	8.03±1.80	6.10±2.82	8.10±1.87	1.242	0.544	0.973

Values presented as mean ± SD; SEM = standard error of the mean.
DM = dry matter; GL = glyphosate; Low-GL = 4 mg GL/kg DM; High-GL = 8 mg GL/kg DM); Trt = treatment; L= linear.

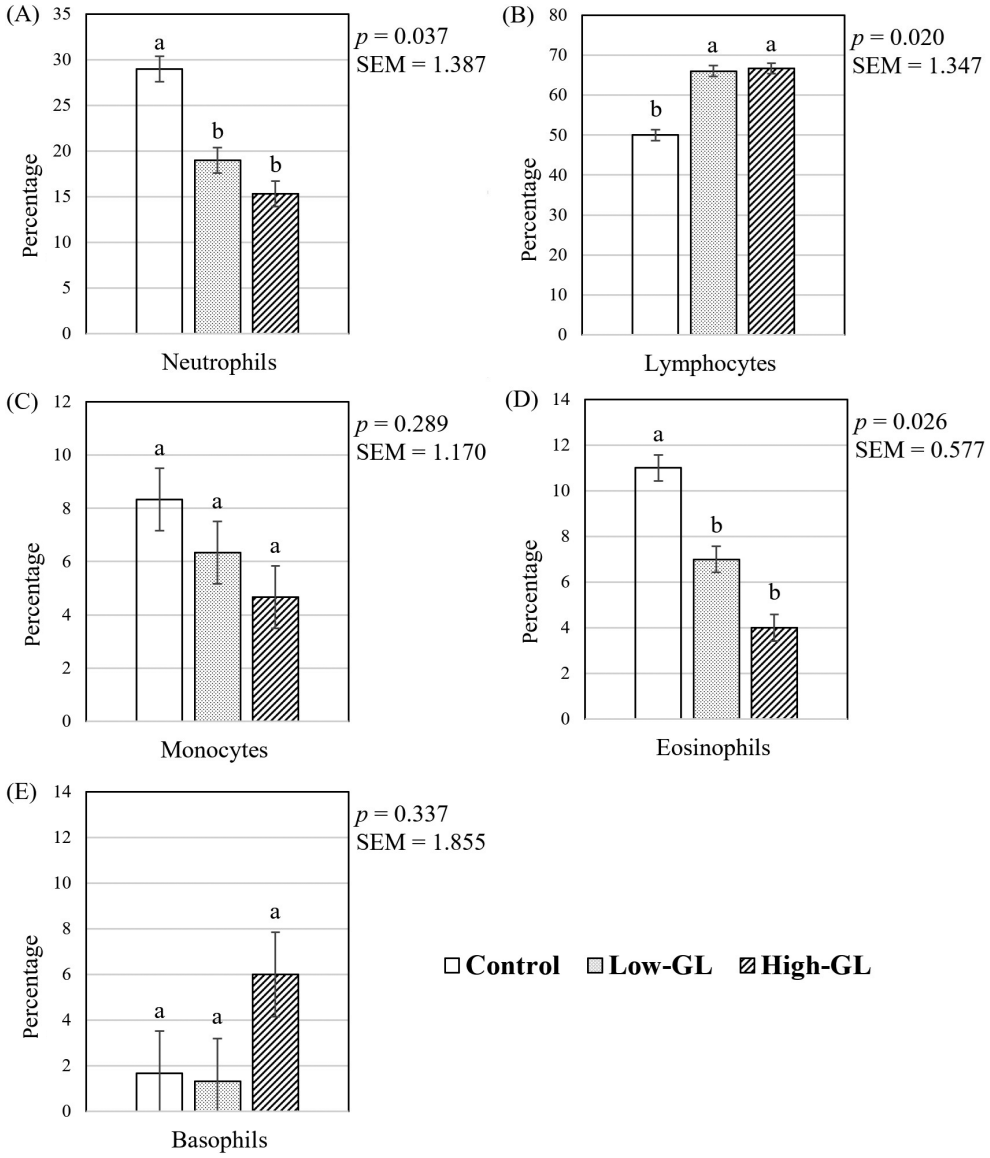

Fig. 1 Effects of feed-contaminated glyphosate in cows receiving glyphosate at 0 mg/kg dry matter (DM) as the control, 4 mg/kg DM (Low-GL) and 8 mg/kg DM on white blood cell differential count proportions of: (A) neutrophils; (B) lymphocytes; (C) monocytes; (D) eosinophils; (E) basophils, where cows were measured at day 21 in period, error bar = SD, and columns with different lowercase superscripts are significantly ($p < 0.05$) different.

Discussion

Feed intake

Based on the results, the GL contamination in the concentrate diet did not have a significant effect on the DM intake due to relatively low levels of GL (4 mg/kg DM) contamination in feed. This was in accordance with the results of Schnabel et al. (2017), who examined the use of GL in the total mixed ration of dairy cows with low and high ratios of concentrated feed and an average dietary GL content of 79.1 mg/d and reported that GL in the feed had no effect on feed intake. This was the case in the current study for both the Low-GL and High-GL diets. Heymann et al. (2023) reported that the effect of GL contamination in the cattle diet did not affect DM when exposed to GL at an average of 171 µg/kg BW/d. Donkin et al. (2003) stated that there was no difference in the ingested DM because the chemical composition of feed and the coefficient of digestible energy were similar.

Milk production and composition

The High-GL concentrate feed significantly decreased both lactose production per day and solids-not-fat in the milk (Table 3). Microbial activity within the rumen and milk composition were not disturbed because the GL administration level in the diet was relatively low; however, the increased days in milk resulted in a slight decrease in milk yield. Schnabel et al. (2017) found that 79.1 mg/d GL did not affect milk production and composition. However, in the current study, milk yield significantly decreased linearly when the higher level of GL was in the diet (Table 3). The same result was achieved with reduced amounts of lactose production, resulting in decreased solids-not-fat production. High levels of GL contamination in the dairy cows' diets reduced ruminal degradation and reduced volatile fatty acids, with the lower volatile fatty acid level being affected by the decrease in lactogenic precursors (Meeprom et al., 2024). In addition, based on the results from the current study, the linear decrease in nitrogen-free extract resulted in lower starch degradability in the rumen, which was affected by low propionate amount for gluconeogenesis using lactose synthesis in the mammary gland. On the other hand, different dietary energy requirements might have had a large effect on the concentrations of protein, fat, lactose, milk urea nitrogen, somatic cell count and fat-to-protein ratios (Donkin et al., 2003). However, Krüger et al. (2014) reported no definitive reduction in the milk yield of cows fed with GL-contaminated feed. Furthermore, Ackermann et al. (2015) suggested that reduced milk production might be due to abnormal changes in the digestive system microbiota caused by a pathogenic microorganism (*Clostridium botulinum*), which resulted in a decrease in the population of enterococci in the gastrointestinal tract. Rulff et al. (2016) suggested that the effect of GL might be masked by disease in cattle, where for example, downer cow syndrome causes cows to have little urination because of kidney damage affecting the health of animal. However, there was no indication that GL affected rumen bacteria or changes in bacterial populations both fiber degradation and starch degradation (Hüther et al., 2005). Therefore, GL did not affect milk production and composition.

Total tract digestibility

In the current study, the digestibility of DM and nitrogen-free extracts decreased linearly ($p < 0.05$), resulting in the GL inhibiting the shikimic pathway and disrupting the ruminal microbial, reducing the ability for feed degradation (Hüther et al., 2005). This was supported by Meeprom et al. (2024), who reported an increase in GL contamination gas kinetics and *in vitro* DM degradability. However, Hüther et al. (2005), who evaluated the effect of contaminated GL in corn silage at 0.77 g/kg DM on sheep, reported that there was no effect on DM and neutral detergent fiber degradability based on the nylon bag technique. They argued that GL affected the microorganisms required in aromatic amino acid synthesis. Furthermore, using only corn silage as a fiber source impacted the activity of cellulolytic bacteria. However, Brede et al. (2022) reported that using GL in feed and the rumen simulation technique (RUSITEC) at levels of 0.1 mg/L, 1.0 mg/L and 10 mg/L had no effect on rumen protein synthesis and nutrient degradation. Additionally, it has been reported exposure of cattle to high concentrations of GL in different ways may reduce the levels of certain microorganisms, especially in groups that digest fibers, such as *Enterococcus spp*., which may result in dysbiosis in cattle, resulting in increased levels of microorganisms, such as *C. botulinum*, in the rumen of cattle (Ackermann et al., 2015). Schrödl et al. (2014) showed that dairy cows exposed to high levels of GL experienced changes in the fungal composition in the rumen compared to unexposed cattle. Aitbali et al. (2018) concluded that herbicides interfered with the balance of rumen microorganisms.

Nitrogen balance

The chemical name of GL is N-phosphonomethylglycine, which cannot be processed in the rumen, according to Riede et al. (2016), who reported that a diet contaminated with GL decreased the ammonia nitrogen concentration. This could be explained by a decrease in the ruminal degradation of protein, causing increased fecal nitrogen excretion. In addition, the rumen microorganism would lose their function of aromatic amino acid synthesis associated with nitrogen feed (Vivancos et al., 2011). Furthermore, based on the results in the current study, the linear increase in fecal nitrogen was directly linked to the nitrogen intake that had a linear increase. However, Schnabel et al. (2017) reported that there was no effect on ammonia nitrogen in dairy cows receiving Roundup Record® at 80 mg/d. However, the impact on microbial protein synthesis has not been clear as there has been no published research on nitrogen utilization by ruminal microorganisms and the nitrogen balance from cows fed GL herbicide.

Blood profiles

GL has an effect on gall bladder weight. For example, Braun (2009) reported that the gall bladder wall thickness in cattle could be inflamed by GL. Later, Heymann et al. (2023)

observed that γ-glutamyltransferase in bovine blood serum was an inflammatory marker; in particular, total leukocytes were affected after 15 wks in cattle subjected to GL exposure. The current study showed that the neutrophil and eosinophil percentages decreased, indicating that adding GL to the cows' diet was related to inflammation, a common cause of infection. A decrease in the white blood count can be due to many factors, including exposure to medicine, chemicals and X-rays (Mosley et al., 2024). A study by Schnabel et al. (2020) reported that the dietary use of GL at 132.8 µg/kg body weight did not affect the counts of white blood cells and red blood cells. However, hematopoietic changes might directly result from GL toxicity in conjunction with increased exposure to different concentrations and durations. Heymann et al. (2023) showed that the daily intake of GL of 171 µg/kg BW/d in dairy cow diets did not affect the different types of red blood cells; however, these cells could be affected by the proportions of concentrate and coarse feed in association with intake duration. It has also been reported that the decrease in red blood cell and hemoglobin levels is caused by the production of reactive oxygen species induced by herbicide Roundup exposure. Herbicide Roundup has been reported to disrupt the body's metabolism by destroying antioxidant defences leading to lipid peroxidation and changes in biochemical and hematological parameters of exposed animals (Owagboriaye et al., 2019). Deshmukh et al. (2013) concluded that GL use at 4,000 mg caused an increase in white blood cells, red blood cells and hemoglobin. In the current study, Low-GL and High-GL contamination in the concentrate at concentrations of 4.90 mg/kg and 6.70 mg/kg, respectively, resulted in a linear decrease in the neutrophil and eosinophil levels. However, GL contamination in the concentrate at the same levels resulted in linear lymphocyte increases ($p < 0.05$). Schnabel et al. (2020) in their study on dairy cows, reported that dietary GL did not affect white blood cell counts, but did impact on eosinophils, which fluctuated over time. In addition, Prasad et al. (2009) studied the GL effect in Swiss white mice of concentrations of 25 mg/kg and 50 mg/kg body weight. They reported an abnormality in the chromosomes of spinal cord cells causing a decrease in red blood cells. However, chromosomal alterations and reduced red blood cell counts might have been due to an imbalance of antioxidants and catalytic agents in the bone marrow. In addition, Deshmukh et al. (2013) evaluated GL administration at 4,000 mg in rats and reported an increase in white blood cells.

Overall, based on these reported studies, it can be concluded that a concentrate diet contaminated with GL herbicide at 47.52 mg/d had no effects on milk production, nutrient digestibility, nitrogen balance and blood cell count. However, when a cow received greater exposure to GL herbicides, there were decreases in lactose production, blood neutrophils and lymphocytes. Future research should include studying the effect of heavily sprayed crops or improperly handled feed compared to non-herbicide management and also consider iso-caloric and iso-nitrogenous feed formulations. Furthermore, the sample group must include low-, medium- and high-yielding dairy cows because of their different health conditions. Finally, there should be experiments to test various related factors such as production, days in milk and time to receive.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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