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Excretion pathways and ruminal disappearance of glyphosate and its degradation product aminomethylphosphonic acid in dairy cows

D. von Soosten,* U. Meyer,* L. Hüther,* S. Dänicke,* M. Lahrssen-Wiederholt,† H. Schafft,† M. Spolders,† and G. Breves‡[1]
*Institute of Animal Nutrition, Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health, Bundesallee 50, 38116 Brunswick, Germany
†Federal Institute for Risk Assessment (BfR), Department Safety in the Food Chain, Max-Dohrn-Str. 8-10, 10589 Berlin, Germany
‡Department of Physiology, University of Veterinary Medicine Hannover, Bischofsholer Damm 15, 30173 Hannover, Germany

ABSTRACT

From 6 balance experiments with total collection of feces and urine, samples were obtained to investigate the excretion pathways of glyphosate (GLY) in lactating dairy cows. Each experiment lasted for 26 d. The first 21 d served for adaptation to the diet, and during the remaining 5 d collection of total feces and urine was conducted. Dry matter intake and milk yield were recorded daily and milk and feed samples were taken during the sampling periods. In 2 of the 6 experiments, at the sampling period for feces and urine, duodenal contents were collected for 5 d. Cows were equipped with cannulas at the dorsal sac of the rumen and the proximal duodenum. Duodenal contents were collected every 2 h over 5 consecutive days. The daily duodenal dry matter flow was measured by using chromium oxide as a volume marker. All samples (feed, feces, urine, milk and duodenal contents were analyzed for GLY and aminomethylphosphonic acid (AMPA). Overall, across the 6 experiments (n = 32) the range of GLY intake was 0.08 to 6.67 mg/d. The main proportion (61 ± 11%; ±SD) of consumed GLY was excreted with feces; whereas excretion by urine was 8 ± 3% of GLY intake. Elimination via milk was negligible. The GLY concentrations above the limit of quantification were not detected in any of the milk samples. A potential ruminal degradation of GLY to AMPA was derived from daily duodenal GLY flow. The apparent ruminal disappearance of GLY intake was 36 and 6%. In conclusion, the results of the present study indicate that the gastrointestinal absorption of GLY is of minor importance and fecal excretion represents the major excretion pathway. A degradation of GLY to AMPA by rumen microbes or a possible retention in the body has to be taken into account.

Key words: glyphosate, urine, feces, milk

INTRODUCTION

Glyphosate (*N*-phosphonomethylglycine) is a broad-spectrum, nonselective herbicide and the most used agent in agriculture worldwide for weed control and plant growth regulation (Dill et al., 2010). In 2012, the annual global glyphosate (**GLY**) production volume was 720,000 t and approximately 45% of the global production volume was administered to glyphosate-resistant transgenic plants (i.e., soybean, corn, cotton, canola; Transparency MarketResearch, 2014). For food-producing animals, soybean products are an important protein source, and approximately 75% of genetically modified soybeans are used for feed production (Flachowsky, 2013). Thus, it can be expected that dairy cows are exposed to GLY by consumption of soybean meal from glyphosate-treated soybeans or other GLY-treated crop products. The International Agency for Research on Cancer (**IARC**) re-evaluated the carcinogenicity of GLY and classified GLY as probably carcinogenic to humans (IARC, 2015). From the IARC report, Guyton et al. (2015) deduced a potential role of GLY for development of tumors in kidney and vessels in mice; however, the administered dosages of glyphosate in these mice studies were considerably higher than a possible average daily intake. In dairy cows, GLY has been discussed controversially as a predisposing factor for the development of the so-called chronic visceral botulism, a syndrome with no clear clinical appearance (Krüger et al., 2012, 2013; Germany, 2013; Gerlach et al., 2014; Ackermann et al., 2015). Therefore, it is important to know to what extent GLY is metabolized and absorbed in the gastrointestinal tract of dairy cows. Furthermore, the transfer into milk is important

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[1]Corresponding author: Gerhard.Breves@tiho-hannover.de

for consumer protection. Nothing is known about a potential ruminal degradation of GLY. In the environment, GLY is degraded to aminomethylphosphonic acid (**AMPA**) by soil microbes. The bacterium *Pseudomonas* sp. strain LBr is responsible for this detoxification route of GLY (Jacob et al., 1988). A second route of detoxification of GLY can be mediated by *Pseudomonas* sp. strain PG29829; this bacterium degraded GLY to glycine. However, the detoxification to glycine has been demonstrated to be of minor importance (Jacob et al., 1988). The fate of GLY consumed by dairy cows is currently not known and, therefore, the objective of our study was to investigate ruminal disappearance and to quantify excretion of GLY with feces, urine, and milk.

MATERIALS AND METHODS

Six balance experiments with collection of total urine and feces were conducted at the experimental station of the Institute of Animal Nutrition, Friedrich-Loeffler-Institut, Brunswick, Germany. The experiments were approved by the Lower Saxony State Office for Consumer Protection and Food Safety, Oldenburg, Germany.

Animals, Feeding, and Design of the Experiments

Overall, the 6 experiments included 32 lactating dairy cows of the German Holstein breed. For experiments 1 to 6, we used 5, 6, 5, 4, 6, and 6 animals per experiment, respectively. The animals were, on average, 90 DIM and in their second to fifth lactation. All cows were fitted with rumen and duodenum cannulas and were housed in a tiestall barn. Milking took place twice daily at 0530 and 1530 h. The animals were fed at the milking times. In all experiments the diet was based on maize silage (single forage component) and concentrates in different proportions (Table 1). The composition of the concentrates as well as the GLY and AMPA concentrations in the concentrates are shown in Table 2. Each balance experiment lasted 26 d. The first 21 d were allowed for equilibration to the experimental diet and the remaining 5 d were the sampling period. In experiments 1 and 2, the quantitative collection of urine and feces was followed by the quantification of daily duodenal dry matter flow (**DMF**) for 5 consecutive days.

Table 1. Forage-to-concentrate ratio of the diet during the experiments

Experiment	Maize silage (%)	Concentrate (%)
1	60	40
2	60	40
3	70	30
4	55	45
5	70	30
6	70	30

Measurements and Sample Collection

During the sampling period, DMI and milk yield were recorded in each individual animal daily. Feed samples for maize silage were taken twice and concentrate samples once during the sampling period. Milk samples were taken once at morning and evening milking in the sampling periods. Total collection of feces and urine was conducted over 5 consecutive days. Cows were equipped with urine devices for separated drain of urine. The device was manufactured of artificial leather and was fitted and agglutinated around the vulva and pins. A polyvinylchloride tube drained the urine into a canister. The feces were collected in a stainless steel

Table 2. Composition and concentrations of glyphosate and aminomethylphosphonic acid in the concentrates of the different experiments

	Experiment					
Component (%, unless noted)	1	2	3	4	5	6
Soybean meal	20	20	25	15		
Rape seed meal			13			
Barley grain	22	22		14.7		
Wheat grain	22	22	36.5	29		
Wheat gluten					10	10
Maize grain	18	18		29	35	35
Sugar beet pulp, dried	15	15	18.3	8.4	48	48
Urea	2	2	2.5	1	3	3
Calcium carbonate/dicalcium phosphate			2.5	1.5	1.5	1.5
Sodium chloride			0.2	0.2	0.2	0.2
Mineral and vitamin-mix	1	1	2.0	1.2	2.3	2.3
Glyphosate (mg/kg of DM)	0.95	0.48	0.82	0.08	0.02	0.03
AMPA[1] (mg/kg of DM)	0.65	0.43	0.46	<LOQ[2]	<LOQ	0.02

[1]Aminomethylphosphonic acid (degradation product of glyphosate).

[2]<LOQ = AMPA concentrations in the samples were lower than the limit of quantification (LOQ).