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Effect of High Dietary Nitrate on the Disposition of Sulfamethazine [4-Amino-*N*-(4,6-dimethyl-2-pyrimidinyl)benzenesulfonamide] in Swine†

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Swine (58-74-kg initial weight) were fed a corn-soybean meal basal diet that contained 110 ppm sulfamethazine [4-amino-*N*-(4,6-dimethyl-2-pyrimidinyl)benzenesulfonamide; sulmet] and 0, 10, 100, 500, or 1000 ppm nitrate. The concentrations of nitrite in the oral cavity and the concentrations of desaminosulfamethazine [*N*-(4,6-dimethyl-2-pyrimidinyl)benzenesulfonamide, DA-sulmet] in the blood were increased by feeding the highest levels of nitrate; however, 10 or 100 ppm of nitrate in the diet had little or no effect on nitrite and DA-sulmet concentrations in the oral cavity. Supplementing the diet with all levels of nitrate had little or no effect on the concentrations of sulmet and N^4-Ac-sulmet in swine blood.

Sulfamethazine [4-amino-*N*-(4,6-dimethyl-2-pyrimidinyl)benzenesulfonamide, sulmet; Figure 1] is used extensively by the swine industry to prevent and control bacterial infections and to increase animal growth rate and improve feed efficiency. Concern about tissue residues was the impetus for studies on the disposition of sulmet and related sulfonamide drugs in swine. When [^{14}C]sulmet was administered orally to swine, most of the radioactivity was excreted in the urine as sulmet and N^4-acetylsulfamethazine (N^4-Ac-sulmet; Figure 1) (Paulson et al., 1981). A unique metabolite, desaminosulfamethazine [*N*-(4,6-dimethyl-2-pyrimidinyl)benzenesulfonamide, DA-sulmet; Figure 1] was also present in the blood and other tissues from swine dosed with [^{14}C]sulmet (Paulson and Struble, 1980; Paulson et al., 1981). Subsequent studies with rats demonstrated that high dietary nitrite greatly enhanced the conversion of sulmet to DA-sulmet (Paulson, 1986) and the deamination of other sulfonamide drugs (Woolley and Sigel, 1982; Nelson et al., 1987). Later it was unequivocally shown that sulmet in the presence of nitrite and acid conditions in the stomach of the rat was converted to a diazonium cation (DZ-sulmet; Figure 1) and that this reactive intermediate was a precursor to DA-sulmet (Paulson et al., 1987).

Observations that DZ-sulmet was weakly mutagenic when evaluated by the Ames test (Paulson et al., 1987) and that the half-life of DA-sulmet in swine tissues was several times longer than the half-life of sulmet (Mitchell and Paulson, 1986) were the stimulus for additional investigations to determine the effect of dietary nitrite on the disposition of sulmet in swine (Paulson and Feil, 1987). Comparative studies in which swine were given a single oral dose of [^{14}C]sulmet in combination with nitrite (165 mg of [^{14}C]sulmet and 2.25 g of $NaNO_2$ in 1.5 kg of feed) or with [^{14}C]-DZ-sulmet alone (165 mg of sulmet equiv in 1.5 kg of feed) provided evidence that sulmet in the presence of nitrite and the acid conditions in the gastrointestinal tract of swine was converted to DZ-sulmet and that DZ-sulmet was a precursor to DA-sulmet (Figure 1) (Paulson and Feil, 1987). Nitrite supplementation decreased the concentration of sulmet and increased the concentration of DA-sulmet in the swine blood.

The levels of nitrite used in the experiments described above were in excess of the levels expected in conventional corn-soybean meal swine diets. However, high concentrations of nitrate are sometimes present in feed and H_2O consumed by swine (Wright and Davison, 1964), and there is much evidence that nitrate is reduced to nitrite by microbes in the oral cavity and, under certain condi-

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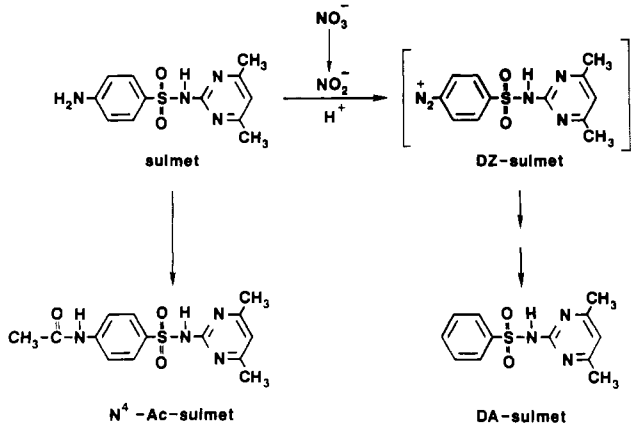

Figure 1. Disposition of sulmet and related compounds in animals.

tions, in the stomach and urinary tract of some animals (Hartman, 1982). After swine were given a single oral dose of [^{14}C]sulmet in combination with nitrate (165 mg of [^{14}C]sulmet and 2.06 g of $NaNO_3$ in 1.5 kg of feed), the concentration of [^{14}C]-DA-sulmet in the blood was slightly higher than the concentration of [^{14}C]-DA-sulmet in the blood of swine given only [^{14}C]sulmet (Paulson and Feil, 1987). Although the effect was small, it suggested that under other conditions high dietary nitrate could more profoundly alter the disposition of sulmet. For instance, continuous feeding of rations and/or water containing large amounts of nitrate to swine could induce microbial nitrate reductase activity in the oral cavity of these animals; if this were to occur, the formation of nitrite and the diazotization of sulmet would be expected to increase. Thus, the studies reported here were initiated to determine the effect of feeding nitrate-fortified diets for 3 weeks on the disposition of sulmet in swine.

MATERIALS AND METHODS

Thirty crossbred swine, weighing from 58 to 74 kg, were randomly assigned to five treatments (six animals per treatment) and fed a basal diet supplemented with varying amounts of nitrate for 21 days. Animals in treatment I (controls) were fed the basal diet consisting of 80.8% corn, 16.5% soybean meal, 1% limestone, 1% dicalcium phosphate, 0.3% trace mineralized salt, and 0.4% vitamin premix. The trace mineralized salt contained 0.35% Zn, 0.28% Mn, 0.175% Fe, 0.035% Cu, 0.007% I, and 0.007% Co. The vitamin premix contained 182 000 USP units of vitamin A, 18 200 USP units of vitamin D_3, 182 mg of riboflavin, 593 mg of *d*-pantothenic acid, 1454 mg of niacin, 3636 mg of choline chloride, and 1.45 mg of vitamin B_{12}/kg. The basal diet was also supplemented with 176 mg of ZnO and 110 mg of sulmet/kg. The basal diet was fortified with $NaNO_3$ to provide diets for treatments II–V containing the following concentrations of supplemental nitrate: II, 10 ppm; III, 100; IV, 500; V, 1,000. The amount of Na in the diets was equalized by adding appropriate amounts of NaCl. All animals were fed and watered ad libitum, and pens were cleaned daily to minimize the exposure of animals to excreta. Animal weights and nitrite in the oral cavity were determined when the experiment was initiated and at 1, 2, and 3 weeks thereafter. A swab was used to collect saliva from the oral cavity (Woolley and Sigel, 1982). A blood sample was collected (anterior vena cava) from each animal at the conclusion of the experiment. The animals were lightly anesthetized with a mixture of halothane and nitrous oxide to facilitate the blood collection process.

Blood samples were analyzed for sulmet, N^4-Ac-sulmet, and DA-sulmet as previously described (Paulson et al., 1985). Briefly, this procedure involved methanol extraction, hexane–water partitioning, XAD-2 column chromatography, C-18 reversed-phase chromatography, and comparison of the UV response (peak height) to the response due to known amounts of reference compounds.

Nitrite in the oral cavity was measured by the method of Woolley and Sigel (1982) except that nitrate was not added to the reaction mixture. The pink resulting from the formation of the sulfanilamide–*N*-1-naphthylethylenediamine complex was assigned a numerical score on the basis of color development after 15 min. The color scores were as follows: 0, no color; 0.5, trace; 1.0, faint pink; 2.0, moderate pink; 3.0, deep pink. The color development was scored by two individuals, and the scores were averaged.

RESULTS AND DISCUSSION

All animals in all treatment groups appeared healthy and vigorous throughout the 3-week treatment period. The average daily weight gains by the five groups of animals were similar (range 0.9–1.0 kg/day; Table I).

The concentrations of nitrite in the oral cavity of swine increased when rations containing 500 and 1000 ppm nitrate were fed; however, the lower levels of nitrate supplementation had little or no effect. The concentrations of nitrite in the oral cavity of all groups of swine on the 7th and 14th day of the experiment (data not shown) were essentially identical with those present on the 21st day of the experiment; thus, steady-state levels were apparently established in 1 week or less.

The concentrations of DA-sulmet in the blood of swine fed the diet containing 500 and 1000 ppm supplemental nitrate were elevated (2.9 and 6.3 ppm) in comparison to the concentrations of DA-sulmet in the blood of unsupplemented controls (1.1 ppm); however, 10 and 100 ppm of nitrate supplementation had relatively little or no effect on DA-sulmet concentrations in the blood (Table I). Supplementing the swine ration with low, intermediate, and high levels of nitrate had no effect on the concentrations of sulmet and N^4-Ac-sulmet in the animal's blood (Table I).

These studies demonstrated that the highest level of supplemental nitrate increased the amount of nitrite in the oral cavity. When this occurred, there was a concomitant increase in the concentration of DA-sulmet in the blood as expected—based on the results of previous studies (Paulson, 1986; Paulson et al., 1987; Paulson and Feil, 1987). Those studies provided unequivocal evidence that sulmet in the presence of nitrite in the gastrointestinal tract was converted to DA-sulmet. However, the percentage of the nitrate supplement that was reduced to

Table I. Effect of Supplementing the Diet with Nitrate on Growth Rate, Nitrite in Oral Cavity, and Concentration of Sulmet, N^4-Ac-Sulmet, and DA-Sulmet in the Blood[a]

item	nitrate supplement in diet, ppm				
	0	10	100	500	1000
av daily gain, kg	0.98 ± 0.13	0.92 ± 0.07	0.97 ± 0.10	0.87 ± 0.14	1.0 ± 0.19
nitrite in oral cavity, color score[b]	0.1 ± 0.2	0.2 ± 0.3	0.7 ± 0.4	1.8 ± 0.8	2.2 ± 0.8
sulmet in blood, ppm	8.0 ± 2.6	6.9 ± 1.3	7.3 ± 1.0	7.1 ± 2.4	6.4 ± 1.8
N^4-Ac-sulmet in blood,[c] ppm	1.4 ± 0.3	1.1 ± 0.5	1.3 ± 0.5	1.0 ± 0.3	1.1 ± 0.6
DA-sulmet in blood,[c] ppm	1.1 ± 0.6	1.3 ± 1.0	1.8 ± 0.8	2.9 ± 0.5	6.3 ± 3.0

[a] All values shown in this table are for measurements made on the 21st day of the experiment (mean ± standard deviation). [b] Color score: 0 = no color; 0.5 = trace; 1.0 = faint pink; 2.0 = moderate pink; 3.0 = deep pink. [c] Expressed as ppm of sulmet equiv.

nitrite in the studies reported here was apparently small. If a large percentage of the nitrate supplement would have been reduced to nitrite, a greater increase in the concentration of DA-sulmet in the blood would be expected. For example, in previous studies approximately 71% of the ^{14}C activity in the blood was present as [^{14}C]-DA-sulmet 24 h after swine were fed 1.5 kg of feed containing 110 ppm of [^{14}C]sulmet and 1000 ppm of nitrite (Paulson and Feil, 1987). Six hours after rats were fed a meal containing 100 ppm [^{14}C]sulmet and 0, 10, 100, or 1000 ppm of nitrite, the percentages of ^{14}C activity in the blood that was present as [^{14}C]-DA-sulmet were 0.6, 1.2, 5.1, and 32.6, respectively (Paulson, 1986).

The studies reported here also demonstrated that the concentrations of sulmet and N^4-Ac-sulmet in the blood were not altered by even the highest level of nitrate supplementation. This supports the conclusion that only a small proportion of the nitrate supplement was reduced to nitrite. When swine were given a 1.5-kg meal containing 110 ppm of [^{14}C]sulmet and 1000 ppm nitrite, the concentrations of both sulmet and N^4-Ac-sulmet in blood were dramatically lowered (Paulson and Feil, 1987).

These observations are important as they relate to the effectiveness of sulmet used as an antibiotic in animal production. The highest level of nitrate supplementation (1000 ppm) tested in these studies is in excess of the levels of nitrate normally present in commercial swine diets (Wright and Davison, 1964). Thus, it is unlikely that the nitrate usually present in swine diets significantly alters the effectiveness of sulmet when used as an antibiotic and growth-promoting agent in commercial swine production.

Registry No. NO_3^-, 14797-55-8; NO_2^-, 14797-65-0; sulfamethazine, 57-68-1; deaminosulfamethazine, 6149-31-1; N^4-acetylsulfamethazine, 100-90-3.

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Mobility of Two Sulfonylurea Herbicides in Soil

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Intact field soil cores were used to compare the mobility of DPX-A7881 [2-[[[[[4-ethoxy-6-(methylamino)-1,3,5-triazin-2-yl]amino]carbonyl]amino]sulfonyl]benzoate], a new sulfonylurea herbicide, and chlorsulfuron [2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide] in four soils. DPX-A7881 was generally less mobile than chlorsulfuron in soil. This was explained on the basis of the reduced water solubility of DPX-A7881 relative to chlorsulfuron (1.7 and 300 ppm, respectively, at pH 5.0). Enhanced mobility of both herbicides in soil was related to higher soil pH and low organic matter content.

DPX-A7881, a new sulfonylurea herbicide, is currently being evaluated for selective broadleaf postemergence weed control in non-triazine tolerant canola, such as *Brassica campestris* L. Tobin and *Brassica napus* L. Westar, for which no herbicide is presently registered in Canada.

The movement of a herbicide through the soil profile has important implications with respect to its efficacy in

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