

# Effect of feed particle size and dietary melatonin supplementation on gastric ulcers in swine

H. L. Ayles<sup>1</sup>, R. M. Friendship<sup>2</sup>, G. A. Bubenik<sup>3</sup>, and R. O. Ball<sup>4,5</sup>

<sup>1</sup>Department of Animal and Poultry Science, <sup>2</sup>Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada N1G 2W1; <sup>3</sup>Department of Zoology, University of Guelph, Guelph, Ontario, Canada N1G 2W1; and <sup>4</sup>Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2P5. Received 25 June 1998, accepted 11 February 1999.

Ayles, H. L., Friendship, R. M., Bubenik, G. A. and Ball, R. O. 1999. **Effect of feed particle size and dietary melatonin supplementation on gastric ulcers in swine.** *Can. J. Anim. Sci.* **79**: 179–185. Gastric ulcers may be an underrecognized problem in the swine industry. An experiment was conducted to evaluate the effects of dietary melatonin supplementation and particle size on prevalence and severity of ulcers in growing pigs. Sixty-four Yorkshire barrows and gilts (29.5 ± 1.0 kg) received either a finely ground (763 ± 2.18 µm) or a coarsely ground (953 ± 2.29 µm) corn–wheat diet, either with or without added melatonin (5 mg kg<sup>-1</sup>). One half of the pigs underwent endoscopic examination to assess the severity of ulcers on days 4 and 25. Growth performance and feed consumption were monitored throughout the experiment. All pigs were slaughtered on day 28, and ulcers were assessed post-mortem. Endoscopy at the beginning of the experiment found that 53% of the pigs already had some degree of ulceration. Pigs fed the fine diet had greater average daily gain and average daily feed intake than pigs fed the coarse diet ( $P < 0.05$ ), but particle size did not significantly affect prevalence of stomach ulcers. Melatonin supplementation increased the apparent digestibility of crude protein ( $P < 0.04$ ) and dry matter ( $P < 0.08$ ) of the diets and decreased the prevalence of ulcers ( $P < 0.05$ ) but did not significantly affect growth performance. Pigs that received melatonin had higher levels of melatonin ( $P < 0.01$ ) in most gastrointestinal tissues, with highest concentrations found in the stomach ( $P < 0.001$ ). Pigs that did not have ulcers had a higher concentration of melatonin in the plasma and stomach than pigs with ulcers ( $P < 0.05$ ). Melatonin may be useful in decreasing ulcers. Initial ulcer condition may be a significant source of variation in growth experiments.

**Key words:** Pigs, gastric ulcers, particle size, melatonin, gastrointestinal tract

Ayles, H. L., Friendship, R. M., Bubenik, G. A. et Ball, R. O. 1999. **Effet de la granulométrie des particules alimentaires et d'un complément de mélatonine sur l'apparition des ulcères gastriques chez les porcs.** *Can. J. Anim. Sci.* **79**: 179–185. Les ulcères gastriques pourraient bien être un problème trop peu connu dans le secteur porcin. Nous avons réalisé une expérience pour évaluer les effets d'un complément de mélatonine dans l'alimentation et du calibre des particules alimentaires sur la fréquence et sur la gravité des ulcères chez les porcs en croissance. Soixante-quatre porcs Yorkshire, castrats et cochettes, d'un poids moyen de 29,5 kg (± 1,0 kg) ont reçu un aliment maïs-blé, moulu fin (763 ± 2,18 µm) ou gros (953 ± 2,29 µm) et complété ou non de mélatonine à la dose de 5 mg kg<sup>-1</sup>. La moitié des animaux subissaient un examen endoscopique pour évaluer la gravité des ulcères au jour 4 et au jour 25 de l'expérience. Les performances de croissance et la consommation alimentaire étaient surveillées en permanence. Tous les porcs étaient abattus au jour 28, après quoi on évaluait la gravité des ulcères. L'endoscopie faite au début de l'expérience a révélé que 53% des bêtes souffraient déjà d'ulcères plus ou moins avancés. Les porcs recevant l'aliment fin manifestaient un GMQ et un taux d'ingestion corrigé plus élevé que ceux recevant l'aliment moulu gros ( $P < 0,05$ ), mais la granulométrie n'avait que relativement peu d'effets sur la gravité des ulcères. La complémentation de mélatonine produisait une hausse de la digestibilité apparente de PB ( $P < 0,04$ ) et de m.s. ( $P < 0,08$ ) des aliments et une réduction de la gravité des ulcères ( $P < 0,05$ ), mais elle avait peu d'effet sur les performances de croissance. Les porcs recevant la mélatonine manifestaient des niveaux plus élevés de cette substance ( $P < 0,01$ ) dans la plupart des tissus gastro-intestinaux, les plus fortes concentrations se retrouvant dans l'estomac ( $P < 0,001$ ). Les porcs sans ulcères avaient une plus forte concentration de mélatonine dans le plasma et dans l'estomac que les bêtes souffrant d'ulcères ( $P < 0,05$ ). La mélatonine pourrait donc être utile pour réduire la gravité des ulcères. À noter que l'état initial de l'animal relativement aux ulcères peut être une cause significative de variabilité dans les expériences de croissance.

Gastric ulcers are a significant problem in the swine industry. In some herds, mortality due to ulcers can reach 1% or higher, with losses due to culling up to 3% (Nielson 1995). Studies conducted on slaughter pigs revealed that the prevalence of erosions in the pars esophagea of the stomach can

be high; for example, 32% (Christensen and Cullinane 1990), 65% (Straw et al. 1992), 89% (Elbers et al. 1995), 99% (Driesen et al. 1987), and 100% (Ito et al. 1974). A small dietary particle size has been implicated in an

**Abbreviations:** ADG, average daily gain; CF, crude fibre; CP, crude protein; DM, dry weight; GE, gross energy; GLM, general linear model; LSD, least-significant difference; LSM, least-squares mean; ME, metabolizable energy; SBM, soybean meal; SEM, standard error of the mean

<sup>5</sup>Author to whom correspondence should be addressed. Tel: (403) 492-7151, fax: (403) 492-9130, e-mail: rball@afns.ualberta.ca.

increased risk of gastric ulcers in swine (Maxwell et al. 1972; Hedde et al. 1985; Wondra et al. 1995). However, smaller particle size of the diet is also related to improved growth performance in pigs (Hedde et al. 1985).

Melatonin, a hormone produced in the gastrointestinal tract, may be useful in the prevention of gastric ulcers in swine (Ayles et al. 1996a). Studies using rodents indicated that melatonin may be involved in the regulation of the motility of the gastrointestinal tract and in the ulceration process (Bubenik and Dhanvantari 1989; Cho et al. 1989; Khan et al. 1990).

Gastric ulcers in swine are usually assessed through post-mortem examinations, although endoscopic examination in vivo has been reported in a few studies within the veterinary literature (Kowalczyk et al. 1968; Korue et al. 1981; Ayles et al. 1996b; Mackin et al. 1997). Initial ulcer condition has seldom been previously considered as a variable in ulcer research with swine. Ulcers can occur quite rapidly in young pigs (Ayles et al. 1996b). Therefore, it may be necessary to know ulcer status at the beginning, as well as the end, of an experiment. We employed a modern flexible endoscope to visualize the gastric lesions (Mackin et al. 1997) and thus assess the effects of initial ulcer condition in an experiment with randomly allocated pigs.

The purpose of this experiment was to investigate the effect of dietary particle size and melatonin supplementation on ulcer development, and whether a flexible videoscope could be used to efficiently monitor the prevalence and incidence of ulcers of the pars esophagea in a large group of pigs.

## MATERIALS AND METHODS

### Animals and Diets

All animal procedures were approved by the University of Guelph Animal Care Committee for adherence to the guidelines of the Canadian Council on Animal Care. A total of 64 Yorkshire barrows and gilts were randomly assigned to eight pens (four barrows and four gilts per pen). Pigs were introduced to the experiment at  $29.5 \pm 1.0$  kg (mean  $\pm$  SEM) and individually weighed at the beginning of the trial and every week thereafter. Pigs were given ad libitum access to a pelleted corn-wheat-soy-based grower diet (Table 1). Pens were randomly assigned to a factorial arrangement of treatments for diet particle size and melatonin (Sigma Chemical, St. Louis, MO) supplementation ( $5 \text{ mg kg}^{-1}$ ). The finely ground diet was ground twice with a 3/16-inch screen, and the coarsely ground diet was ground only once with the same screen. Feed consumption and feed efficiency were calculated on a per-pen basis.

### Particle-Size Determination

Particle size of the diet was determined by the method of the American Society of Agricultural Engineers (ASAE 1983). Briefly, a 100-g sample of ground feed (sampled prior to pelleting) was placed in a shaker consisting of 15 brass sieves descending in screen size. All material left on each screen was weighed and entered into the logarithmic equation (ASAE 1983) for particle-size determination. Particle-

**Table 1. Ingredients and analyzed composition of the diet**

Item	%
Corn	40.0
Wheat	31.55
Wheat shorts	9.35
SBM (48% CP)	12.73
Meat meal	1.48
Lignosol	1.25
Calcium carbonate	0.97
Dicalcium phosphate	0.81
Plain salt	0.42
Animal fat	0.50
Choline chloride	0.04
Copper sulfate	0.05
L-lysine (HCl)	0.10
Mineral premix <sup>z</sup>	0.50
Vitamin premix <sup>y</sup>	0.25
Composition (calculated)	
ME (MJ kg <sup>-1</sup> ) <sup>x</sup>	13.22
Composition (analyzed) (% DM) <sup>w</sup>	
GE (MJ kg <sup>-1</sup> )	14.64
DM	86.24
CP	18.99
Fat (ether extract)	3.86
Ash	5.38
CF	3.82
Calcium	1.0
Phosphorus	0.74
Sodium	0.34

<sup>z</sup>Supplied per kilogram diet: 15 mg copper, 100 mg zinc, 100 mg iron, 20 mg manganese, 0.3 mg iodine, 0.3 mg selenium, 1000 ppm titanium oxide, 5 or 0 mg melatonin.

<sup>y</sup>Supplied per kilogram diet: 6000 IU vitamin A, 600 IU vitamin D<sub>3</sub>, 24 IU vitamin E, 1.3 mg menadione, 300 mg choline, 9 mg pantothenic acid, 3 mg riboflavin, 1.2 mg folic acid, 15 mg niacin, 0.9 mg thiamin, 0.9 mg pyridoxine, 120 µg biotin, and 15 IU vitamin B<sub>12</sub>.

<sup>x</sup>Calculated from published values for the diet ingredients from National Research Council (1988) for swine.

<sup>w</sup>Analyzed by methods in Association of Official Analytical Chemists.

size analysis showed that the fine diet had a geometric mean ( $\pm$  geometric variance) particle size of  $953 \pm 2.29 \mu\text{m}$ .

### Endoscopic Examination

One half of the pigs underwent endoscopic examination to determine ulcer condition at the beginning of the trial. Endoscopic examination was planned for day 1; however, demands for clinical use of the endoscope by the veterinary hospital prevented assessment until day 4. Feed was removed from all the pens 12 h before endoscopic examination because an empty stomach is required for visualization of the pars esophageal area. Water was freely available until pigs were removed from pens to be anaesthetized. Endoscopic examination was performed as described elsewhere (Mackin et al. 1997). Briefly, anaesthetic induction was performed with  $30 \text{ mg kg}^{-1}$  intravenous pentobarbital (Somnotol; MTC Pharmaceutical, Cambridge, ON) administered via a butterfly catheter into a marginal ear vein. Further intravenous pentobarbital was given as required to maintain a moderate depth of anaesthesia throughout the endoscopic procedure. Total time of anaesthesia was approximately 15 min. Endoscopic examination was per-

formed with a forward-viewing flexible videoscope with a working length of 100 cm and an outer diameter of 10 mm (GIF type 100 EVIS Gastrointestinal Videoscope; Olympus Corporation, Lake Success, NY) and required approximately 5–7 min pig<sup>-1</sup>. Each endoscopic examination was video-recorded to enable later assessment of ulcer scores. The pars esophageal area was scored from 0 to 3 using the scoring system developed for endoscopy by Mackin et al. (1997) as used by Ayles et al. (1996b); 0, normal; 1, parakeratosis or superficial erosion of < 25% of pars esophageal area; 2, moderate erosion of >25% of pars esophageal area or severe ulceration <10% of pars esophageal area; and 3, severe ulceration >10% of pars esophageal area. On day 25, endoscopy was repeated in the same animals.

### Diet Digestibility

Titanium oxide (1000 ppm) was added to the feed via the mineral premix, as an indigestible marker to determine apparent digestibility of DM and protein. Fecal samples from individual pigs were collected weekly during weighing and at slaughter to determine apparent diet digestibility. The diets and feces were analyzed for percentage of DM, CP and titanium by the methods of the Association of Official Analytical Chemists (1990).

### Plasma Melatonin Analysis

Plasma samples were obtained from the jugular vein immediately postmortem, immediately frozen (–80°C), and stored in air-tight containers in the dark until later analyzed for melatonin concentration by the method of Brown et al. (1985). Briefly, the method involved extraction with dichloromethane, incubation with <sup>3</sup>H-melatonin and melatonin antiserum, separation of the bound melatonin and radioimmunoassay.

### Postmortem Examination

Pigs were slaughtered on day 28. Samples taken were mixed venous blood samples for plasma melatonin, tissue samples from the cardiac region of the stomach, mid-jejunum, mid-ileum and mid-colon for melatonin analysis; and fecal samples for nutrient-digestibility. Stomachs were examined by a veterinary pathologist using a scoring system typical of that used to score ulcers at postmortem (Straw et al. 1992); 0, normal; 1, parakeratosis; 2, focal shallow ulceration; 3, fully developed ulcer.

### Melatonin Analysis of Gastrointestinal Tissues

Samples of the stomach, jejunum, ileum and colon obtained at postmortem were placed in air-tight jars in the dark and frozen (–80°C) until later analyses for melatonin concentration. The tissue sample was homogenized with 10 mL of 0.4 M perchloric acid. The homogenate was centrifuged at 25 000 × g for 10 min. The supernatant was collected into a 50-mL centrifuge tube and adjusted to pH 10 with 4 M KOH. The precipitate was again centrifuged at 25 000 × g for 10 min. Melatonin in the sample was removed via extraction with 15 mL of chloroform (Brown et al. 1985) and then stored at –20°C. Bovine-serum albumin-phosphate buffer (1.25 mL, pH 6.5) was added to each sample, and the

concentration of melatonin was determined by radioimmunoassay (Brown et al. 1985).

### Statistics

The study was designed as a 2 × 2 factorial. Pen was the experimental unit for feed intake and feed conversion, and pig was the experimental unit for all other measures. All analyses were conducted using the GLM procedure of the SAS Institute, Inc. (1985). GLM was used to test for the effects of diet particle size, melatonin treatment, and interaction on ADG, feed-gain, feed intake, diet digestibility, ulcer scores at endoscopy on days 4 and 25 and postmortem, plasma melatonin concentration, and melatonin concentration within the gastrointestinal tract. LSM were calculated and compared by protected LSD. Endoscopic score on day 4 was used as a covariate in the analysis of effects of diet particle size and melatonin treatment on day-25 endoscopy and postmortem ulcer scores. The GLM was performed to test for a relationship between ulcer score and ADG, feed-gain, feed intake, plasma melatonin, and melatonin concentration within the stomach, jejunum, ileum and colon. Agreement between day-25 endoscopy and postmortem ulcer scores was assessed with Cohen's weighted kappa test (Fleiss 1973).

## RESULTS AND DISCUSSION

The particle size of the fine diet used in the present experiment is not as small as used previously by others or by ourselves (Ayles et al. 1996b) to create ulcers. However, in our previous research with a particle size of 578 μm, the pigs developed severe ulcers very rapidly and began to die from gastric hemorrhaging (Ayles et al. 1996b). We believed this was unacceptable and therefore chose the current particle size to produce milder ulcer conditions.

Pigs fed the finely ground diet grew more rapidly than pigs fed the coarse diet ( $P < 0.03$ ) (0.96 vs. 0.79 kg d<sup>-1</sup>) and had a greater feed intake (2.35 vs. 2.25 kg d<sup>-1</sup>) ( $P < 0.001$ ) (Table 2). Particle size did not significantly ( $P > 0.1$ ) affect feed conversion or apparent digestibility of DM and protein.

There were no differences in growth rates, feed conversion or feed intake between pigs supplemented with melatonin and those not supplemented with melatonin (Table 2). However, pigs supplemented with melatonin had better CP ( $P < 0.04$ ) and DM ( $P < 0.08$ ) digestibility than pigs not supplemented with melatonin (Table 2). Melatonin may play a role in gastrointestinal motility (Bubenik et al. 1992; Bubenik and Pang 1994). Bubenik and Pang (1994) suggested that during periods of stress, serotonin will be present in excessive amounts and may affect the function of the gastrointestinal tract. An increase in serotonin concentration has been shown to cause an increase in rate of passage of food and an increase in fecal DM (Bubenik and Dhanvantari 1989), which may reflect a decrease in digestibility. Melatonin has been shown to counteract the effects of serotonin in several studies (Bubenik 1986; Bubenik and Dhanvantari 1989; Cho et al. 1989; Khan et al. 1990). Therefore, the addition of exogenous melatonin may slow the rate of passage of digesta and allow for the absorption of more nutrients, thus improving digestibility. There was a

**Table 2. Growth, feed conversion and apparent diet digestibility for pigs receiving a finely ground diet (763 µm) or coarsely ground diet (953 µm), either with or without melatonin (5 mg kg<sup>-1</sup> diet) for 4 wk**

	Diet <sup>z</sup>		Treatment <sup>y</sup>		Main effects <sup>x</sup>		
	F	C	M	0	D	T	SEM
Number of pigs	32	32	32	32	–	–	–
Initial weight (kg)	29.3	29.7	29.4	29.5	NS <sup>w</sup>	NS	0.53
Final weight (kg)	53.0	51.4	52.2	52.3	NS	NS	0.88
ADG (kg d <sup>-1</sup> )	0.96	0.79	0.88	0.87	0.003	NS	0.02
Feed intake (kg d <sup>-1</sup> ) <sup>v</sup>	2.35	2.25	2.31	2.29	0.001	NS	0.04
Feed/gain ratio (kg kg <sup>-1</sup> ) <sup>v</sup>	2.48	2.86	2.64	2.63	NS	NS	0.19
CP digestibility (%)	72.0	69.8	72.8	69.1	NS	0.04	0.86
DM digestibility (%)	79.6	78.4	80.9	77.9	NS	0.08	0.61

<sup>z</sup>F, fine; C, coarse.

<sup>y</sup>M, melatonin 5 mg kg<sup>-1</sup> diet; 0, no melatonin.

<sup>x</sup>D, diet effect; T, treatment effect. No significant ( $P > 0.10$ ) interactions between diet and treatment were detected.

<sup>w</sup>NS, not significant.

<sup>v</sup>Pen basis, 8 pigs pen<sup>-1</sup>.

**Table 3. Initial mean ulcer score, initial and final ulcer prevalence, and final mean ulcer score for pigs that received a finely ground diet (763 µm) or coarsely ground diet (953 µm), either with or without melatonin (5 mg kg<sup>-1</sup> diet) for 4 wk**

	Diet <sup>z</sup>		Treatment <sup>y</sup>		Main effects <sup>x</sup>			SEM
	F	C	M	0	D	T	TD	
Initial mean ulcer score by endoscopy <sup>w</sup>	1.19	2.0	0.94	2.25	NS <sup>v</sup>	0.05	0.05	0.36
Initial prevalence by endoscopy (%)	37.5	69	31	75	NS	0.05	0.05	0.24
Final mean score by postmortem <sup>u</sup>	1.23	1.41	1.13	1.50	NS	0.02	0.05	0.10
Final mean score by postmortem <sup>t</sup>	1.30	1.95	1.54	1.71	0.01	NS	0.05	0.11
Final mean score by endoscopy <sup>w,t</sup>	2.34	2.38	2.76	2.02	NS	NS	NS	0.32
Prevalence at postmortem (%)	90	81	77.5	93.5	NS	0.05	NS	0.26

<sup>z</sup>F, fine; C, coarse.

<sup>y</sup>M, melatonin 5 mg kg<sup>-1</sup> diet; 0, no melatonin.

<sup>x</sup>D, diet effect; T, treatment effect; TD, interaction.

<sup>w</sup> $n = 32$ , endoscopy ulcer-scoring system: 0, normal; 1, parakeratosis or superficial erosion of <25% of pars esophageal area; 2, moderate erosion of >25% of pars esophageal area or severe ulceration of <10% of pars esophageal area; and 3, severe ulceration >10% of pars esophageal area.

<sup>v</sup>NS, not significant.

<sup>u</sup> $n = 64$ ; ulcers scored at postmortem: 0, normal; 1, parakeratosis; 2, focal shallow ulceration; and 3, fully developed ulcer.

<sup>t</sup> $n = 32$ , initial endoscopic ulcer score used as covariate for analysis of final ulcer scores

<sup>s</sup> $n = 64$ , prevalence is percentage of pigs with ulcers.

tendency  $P = 0.08$ ) for this in the present study; melatonin supplementation increased the apparent digestibility of the protein and DM in the diet by 3.7 and 3.0%, respectively. There were no significant interactions between diet particle size and melatonin treatment for growth performance or apparent DM and protein digestibility.

Severe bleeding ulcers were present in five pigs at the beginning of the experiment. This indicated that ulcers can begin at an early age and may be a significant problem in weanling pigs, as well as in the finisher stage. Pigs had been weaned at approximately 25 d of age and received a simple starter diet (corn-SBM) until entry into the present experiment at about 30 kg. Particle size of the starter diet was not determined.

Endoscopic examination of one half of the pigs at the beginning of the experiment found that, overall, 53% of these pigs already had some degree of ulceration. Random allocation apparently resulted in more pigs with more severe ulcers being allocated to the treatment coarse without melatonin (Tables 3 and 4). However, an early treatment effect due to melatonin cannot be ruled out. Initial ulcer score was subsequently used as a covariate in statistical analysis of

ulcer scores by endoscopy at day 25 and of scores by post-mortem evaluation, as discussed below.

At the initial endoscopy, there was a tendency ( $P < 0.1$ ) for fewer and less severe ulcers in pigs allotted to the fine diet (Table 3). At postmortem, pigs fed the fine diet had final mean ulcer score similar to that of pigs fed the coarse diet. At postmortem, pigs fed the fine diet had a numerically higher prevalence of ulcers (90%) than those fed the coarse diet (81%). There was no difference in final endoscopic ulcer score due to dietary particle size. This is not in agreement with work done by Gamble et al. (1967), Reimann et al. (1968), Pickett et al. (1969), Pocock et al. (1969), Maxwell et al. (1970), and Hedde et al. (1985), who found that a smaller particle size (approximately 500–600 µm) increased both the severity and the incidence of gastric ulcers. A smaller particle size has been shown to increase the pepsin activity, decrease the pH in the esophageal region, reduce the pH gradient between the pars esophageal and pyloric regions, and increase the percent moisture and fluidity of the stomach contents (Maxwell et al. 1972). An increase in fluidity of stomach contents may allow HCl, pepsin and bile acids to come into contact with the unpro-

**Table 4. Comparisons of endoscopic and postmortem ulcer scores for pigs receiving a finely ground diet (763 µm) or coarsely ground diet (953 µm), either with or without melatonin (5 mg kg<sup>-1</sup> diet) for 4 wk<sup>z</sup>**

Diet <sup>y</sup>	Initial endoscopy ulcer score <sup>x</sup>	Final endoscopy ulcer score <sup>x</sup>	Postmortem score <sup>w,v</sup>
C0	2.13a	1.73	1.98a
CM	1.88ab	3.1	1.92a
F0	2.40a	2.73	1.44ab
FM	0b	2.29	1.12b
SEM	0.35	0.23	0.08

<sup>z</sup>A total of 32 pigs were endoscoped, and the same 32 pigs were scored postmortem.

<sup>y</sup>C0, coarse without melatonin; CM, coarse with melatonin (5 mg kg<sup>-1</sup>); F0, fine without melatonin; FM, fine with melatonin (5 mg kg<sup>-1</sup>).

<sup>x</sup>Ulcer-scoring system: 0, normal; 1, parakeratosis or superficial erosion of <25% of pars esophageal area; 2, moderate erosion of >25% of pars esophageal area or severe ulceration of <10% of pars esophageal area; and 3, severe ulceration of >10% of pars esophageal area.

<sup>w</sup>Initial endoscopy ulcer score used as covariate for analysis of diet × treatment effects for final endoscopy and postmortem scores.

<sup>v</sup>Ulcer-scoring system: 0, normal; 1, parakeratosis; 2, focal shallow ulceration; and 3, fully developed ulcer.

a,b Means in columns followed by different letters differ at *P* < 0.05.

tected esophageal area. The lack of difference in ulcer severity due to particle size in the present study may be due to the small number of pigs, short feeding period, or too small a difference in average particle size and particle-size distribution between the diets.

Pigs supplemented with melatonin had a lower initial ulcer score and lower initial prevalence than pigs not supplemented with melatonin (*P* < 0.05) (Table 3). This could have been a random effect, or melatonin may have already had an affect on decreasing ulcer severity by day 4. Those pigs that were supplemented with melatonin had a lower score and prevalence of ulcers at postmortem than those pigs without melatonin supplementation (*P* < 0.05). Khan et al. (1990) found that in stress induced rats, supplementation with melatonin reversed the effects of serotonin on the mucosal blood flow and decreased the severity of gastric ulcers. Melatonin also reduced the severity of ethanol-induced gastric ulcers, apparently by counteracting the effects of serotonin (Cho et al. 1989). Although, the anatomy of the pig's stomach is different than that of the rat's, melatonin may be acting in a similar fashion.

The interactions between diet particle size and melatonin treatment for ulcer scores are shown in Table 4. Initial endoscopy revealed an interaction (*P* < 0.05) because pigs fed the finely ground diet with melatonin had a mean ulcer score of zero, compared with pigs fed finely and coarsely ground diets without melatonin and a coarsely ground diet with melatonin (Table 4). At the final endoscopy, there were no significant differences among finely and coarsely ground diets with and without melatonin for ulcer severity. At postmortem, pigs fed the finely ground diet with melatonin had significantly less severe ulcers (*P* < 0.05) than pigs fed coarsely ground diets with and without melatonin (Table 4), when initial ulcer score was used as a covariate. Ulcer score by day-25 endoscopy and postmortem were numerically different, and the two methods gave slight differences in significance between treatments. However, there was

**Table 5. ADG and feed/gain ratio for pigs with different ulcer severity scores at postmortem**

Ulcer score <sup>y</sup>	0	1	2	3	SEM
Number of pigs	10	31	18	5	
ADG (kg d <sup>-1</sup> ) <sup>x</sup>	0.98a	0.84b	0.88ab	0.91ab	0.02
Feed/gain ratio (kg d <sup>-1</sup> )	2.43	2.88	2.74	2.56	0.21
ADFI (kg d <sup>-1</sup> ) <sup>x,w,v</sup>	2.38	2.41	2.41	2.33	0.04

<sup>z</sup>Initial weight, 29.5 ± 1.0 kg.

<sup>y</sup>Ulcer score at postmortem: 0, normal; 1, parakeratosis; 2, focal shallow ulceration; and 3, fully developed.

<sup>x</sup>Diet used as covariate.

<sup>w</sup>Endoscopic examination used as covariate.

<sup>v</sup>ADFI, average daily feed intake, pen basis.

a,b Means in rows followed by different letters differ at *P* < 0.05.

reasonably good agreement between them for individual pigs (kappa 0.217, variance of kappa = 0.011, *P* < 0.03).

These data show the importance of allocating pigs and treatments based on initial ulcer condition because this may have an important influence on subsequent measures of ulcers. This may explain the many conflicting results in the literature. Pigs are typically allocated to experiments by weight and not by ulcer condition. Future experiments to study the influence of nutritional and management factors on ulcer development should consider both initial observations and allocation to treatments based on endoscopic evaluation of ulcer severity and prevalence.

Ulcer score was significantly related to animal performance (Table 5). Those pigs that had no ulcers at slaughter grew faster than those pigs with a score of 1 (*P* < 0.05) (0.98 vs. 0.84 kg d<sup>-1</sup>), with a tendency (*P* < 0.01) for a lower ADG for all pigs with ulcer scores. This supports work done by Hedde et al. (1985), who found that an increase in ulcer severity was associated with a marked reduction in growth performance. It has been suggested that only pigs with a severe gastric ulcer will have a depressed growth rate, probably due to gastric hemorrhage. The present data suggest that mild lesions may also have a significant economic impact. These results are similar to those of a study in which we fed pigs individually to assess the relationship between ulcers and growth performance (Ayles et al. 1996a,b). The reported high frequency of gastric ulcers in commercial production (Christensen and Cullinane 1990; Straw et al. 1992; Nielson 1995) suggests that lost profit due to ulcers could be considerable.

Melatonin treatment resulted in an increase in melatonin concentration in the gastrointestinal tissues (Table 6), with highest levels in the stomach, followed by the colon, ileum and jejunum. Immunohistologically, Bubenik et al. (1977) found highest levels in the colon and the rectum with intermediate levels in the stomach of the rat. However, by radioimmunoassay, melatonin levels were highest in the stomachs and the colon of rats (Bubenik et al. 1992; Bubenik and Pang 1994). These higher melatonin levels found in the stomach may be an indication of specific receptors in the stomach for melatonin uptake (Bubenik et al. 1993). DeBoer (1988) found higher levels of exogenously administered radioactive melatonin in the ileum and lower levels in the colon in sows; the stomach was not examined.

**Table 6. Effect of dietary melatonin treatment and diet particle size on concentration of melatonin (pg g<sup>-1</sup> tissue) in various regions of the gastrointestinal tract and plasma melatonin (pg mL<sup>-1</sup>)**

	Diet <sup>z</sup>		Treatment <sup>y</sup>		Main effects <sup>x</sup>			
	F	C	M	0	D	T	TD	SEM
Stomach	4869a	3172a	7860a	181	NS <sup>w</sup>	0.0001	NS	636.1
Jejunum	79b	82b	115b	45	NS	0.01	NS	12.3
Ileum	78b	88b	109b	57	NS	0.05	NS	12.6
Colon	326b	305b	574b	117	NS	0.0001	NS	29.3
Plasma melatonin (pg mL <sup>-1</sup> )	89	72	129	32	NS	0.001	0.02	6.8

<sup>z</sup>F, fine; C, coarse.

<sup>y</sup>M, melatonin 5 mg kg<sup>-1</sup> diet; 0, no melatonin.

<sup>x</sup>D, diet effect; T, treatment effect; TD, interaction.

<sup>w</sup>NS, not significant.

a,b Means in columns followed by different letters differ at  $P < 0.001$ .

**Table 7. Relationship between ulcer severity and melatonin concentrations (pg g<sup>-1</sup>) in different regions of the gastrointestinal tract and plasma (pg mL<sup>-1</sup>) for pigs receiving a finely ground diet (763 µm) or coarsely ground diet (953 µm), either with or without melatonin (5 mg kg<sup>-1</sup> diet) for 4 wk**

	0 <sup>z</sup>	1	2	3	SEM
Number of pigs	10	31	18	5	
Stomach <sup>y</sup>	7524.0a	5087.2a	2035.1b	513.7bc	681.2
Jejunum <sup>y</sup>	95.8	87.5	66.1	74.3	13.0
Ileum <sup>y</sup>	102.2	87.0	57.2	123.0	12.7
Colon <sup>y</sup>	323.5	342.9	263.5	123.0	12.7
Plasma	71.5ab	89.0a	64.4b	45.9b	7.01

<sup>z</sup>Ulcer score at postmortem: 0, normal; 1, parakeratosis; 2, focal shallow ulceration; and 3, fully developed ulcer.

<sup>y</sup>Melatonin treatment used as a covariate.

a-c Means in rows followed by different letters differ at  $P < 0.001$ .

This localization of melatonin within the gastrointestinal tract may indicate that melatonin is involved in regulation of gastrointestinal-tract function.

Melatonin concentration in stomach and plasma was related to the prevalence of gastric ulcers in this study (Table 7), even when the influence of melatonin treatment was removed by covariate analysis. Pigs with no ulcers and a score of 1 had very high concentrations of melatonin in the stomach, while pigs with severe ulcers had much lower levels of melatonin in the stomach ( $P < 0.05$ ) (Table 7). Pigs with ulcer scores of 0 and 1 had higher plasma melatonin values than pigs with ulcer scores of 2 and 3. Melatonin synthesized in the gastrointestinal tract has been shown to contribute to systemic circulation, altering plasma melatonin values (Huether 1993; Yaga et al. 1993). Those pigs with severe gastric ulcers may have been producing lower amounts of melatonin in the gastrointestinal tract, specifically in the stomach, where the ulcer is located, and pigs with less severe ulcers may have been producing more melatonin, thus providing a localized protective effect against ulcer formation while also releasing more melatonin into the systemic circulation. The data in Table 4, showing that melatonin treatment reduced ulcer score and prevalence, suggest that a cause and effect relationship may exist. Recent reports support a gastroprotective effect for melatonin (Konturek et al. 1997).

There are very few published data on melatonin metabolism in gastrointestinal tissues, other than descriptive reports. Melatonin synthesis and metabolism appear to be markedly affected by diet and ulcer condition; however, the biological explanations for these effects must await further mechanistic research.

## IMPLICATIONS

Gastric ulceration in swine is not well understood. In addition to lack of knowledge surrounding the cause of gastric ulceration, the effects of ulcers on growth performance of pigs has not been well documented. From this study, it was concluded that pigs with slight ulcerative lesions of the pars esophagea grew significantly slower than pigs that did not have pars esophageal lesions. Mild gastric ulcers may cause pigs to take longer to reach market weight and therefore may inflict economic damage, without clinical signs or mortality. Melatonin may be useful in decreasing the prevalence of gastric ulcers. Endoscopy proved to be a useful tool for assessing ulcers. Initial ulcer condition of pigs may be a significant source of variations in performance and treatment response, not only in ulcer research but also in other nutrition experiments.

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