

The effect of graded levels of melatonin on performance and gastric ulcers in pigs

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¹Department of Animal and Poultry Science, ³Department of Population Medicine, and ⁴Department of Zoology, University of Guelph, Guelph, Ontario, Canada N1G 2W1. Received 24 October 1995, accepted 19 June 1996.

Ayles, H. L., Ball, R. O., Friendship, R. M. and Bubenik, G. A. 1996. **The effect of graded levels of melatonin on performance and gastric ulcers in pigs.** *Can. J. Anim. Sci.* **76**: 607–611. Graded concentrations of dietary melatonin were fed to examine the effects on performance and gastric ulcers in pigs. Four levels of melatonin, 0, 2.5, 5, and 10 mg kg⁻¹, were fed in a finely ground, corn-wheat based diet (578 ± 1.89 µm), designed to induce gastric ulcers, to 64 Yorkshire barrows and gilts between 22.2 ± 2.0 and 102.5 ± 4.3 kg. At postmortem, ulcers were scored from 0 (no ulcer) to 3 (severe ulcer). Volume, percent moisture, pH, and bile acid concentration of the stomach digesta were determined. Melatonin supplementation was associated with a decreasing severity of ulcers ($P < 0.05$). There were no differences in average daily gain between melatonin treatments, although 10 mg kg⁻¹ diet caused a reduction in feed intake. Bile acid concentration in the stomach digesta was highest in pigs receiving the control diet without melatonin ($P < 0.05$) and was increased with increasing ulcer severity ($P < 0.05$).

Key words: Swine, ulcer, diet, melatonin, bile, acids

Ayles, H. L., Ball, R. O., Friendship, R. M. et Bubenik, G. A. 1996. **Effet de niveaux gradués de mélatonine sur les performances zootechniques ainsi que sur l'apparition d'ulcères gastriques chez les porcs.** *Can. J. Anim. Sci.* **76**: 607–611. Nous avons examiné les effets d'administrations graduées de mélatonine dans l'aliment sur les performances zootechniques et sur l'apparition d'ulcères gastriques chez les porcs. Quatre niveaux de mélatonine: 0, 2,5, 5 et 10 mg kg⁻¹ étaient servis à 64 castrats et cochettes à partir du poids vif de 22,2 ± 2,0 à celui de 102,5 ± 4,3 kg, dans un régime maïs-blé moulu fin (578 ± 1,89 µm) conçus pour provoquer l'apparition d'ulcères étaient notés de 0 (absence d'ulcère) à 3 (l'ulcère grave). On mesurait le volume, la teneur en eau, le pH et les concentrations d'acides biliaires du digesta stomacal. L'apport de mélatonine donnait lieu à une baisse de la gravité des ulcères ($P < 0,05$). Il n'y avait pas de différence quant au GMQ et les traitements à la mélatonine, bien qu'à la dose de 10 mg kg⁻¹ d'aliment on observait une diminution de la prise alimentaire. Les concentrations d'acides biliaires dans le digesta stomacal étaient plus fortes chez les porcs recevant l'aliment témoin, sans mélatonine ($P < 0,05$) et, en outre, elles étaient reliées positivement ($P < 0,05$) avec le degré de gravité des ulcères.

Mots clés: Porc, ulcère, régime alimentaire, mélatonine, acides biliaires

Dietary particle size has been implicated as a major factor in oesophagogastric ulcers in swine. A smaller particle size has been shown to increase the incidence and severity of gastric ulcers (Mahan et al. 1966; Maxwell et al. 1967, 1970, 1972; Pickett et al. 1969; Hedde et al. 1985). A smaller dietary particle size may affect the prevalence of ulcers due to an increase in pepsin activity and increased acidity in the stomach contents. Maxwell et al. (1972) found that pH values of the stomach contents decreased from 4.9 to 3.9 in the oesophageal region, while pepsin activity increased from 1 unit mL⁻¹ to 10 units mL⁻¹ when finely ground feed was fed compared with coarse feed. The percent moisture of the stomach contents of the pigs fed fine diets was 75% compared with 63% for those fed coarse diets. A pH gradient, between the cardiac and pyloric regions within the stomach, occurring when a coarse diet was fed but not when a fine diet was fed, indicated that increased mixing had occurred due to the increased fluidity in the stomach of pigs fed the fine ground diets (Maxwell et al. 1972).

A hormone suspected to be involved in the development of ulcers is serotonin (Cho et al. 1989; Bubenik and Dhanvantari 1989; Wong et al. 1990; Tsukamoto et al. 1991). Serotonin is produced in large amounts in the gastrointestinal tract and causes a reduction in mucosal blood flow, increases the sensitivity of the mucosal membrane to gastric acid and bacterial infection, and causes contraction of smooth muscles in the gastrointestinal tract (Quastel and Rahamimoff 1965; Fioretti 1974; Bubenik 1986; Bubenik and Dhanvantari 1989; Cho et al. 1989). Melatonin is also produced in the gastrointestinal tract and has been shown to be an inhibitor of the actions of serotonin (Bubenik 1986). Melatonin reduced the severity of gastric ulcers in stressed rats, possibly by counteracting the effects of serotonin on the gastric mucosal blood flow (Khan et al. 1990). Melatonin may also act by decreasing the tone of the gut muscles, thus slowing down the rate of passage of digesta leading to increased digestibility (Bubenik and Dhanvantari

Abbreviations: ADG, average daily gain; CP, crude protein; DM, dry matter; GU, gastric ulcer

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1989). Excessive contractions of the stomach have been reported to be important in gastric ulcer development, thus melatonin may be important in prevention of ulcer formation (Cho et al. 1989).

Ayles et al. (1995) reported that dietary melatonin supplementation (5 mg kg⁻¹) to pigs from 22 to 52 kg reduced ulcer severity and prevalence. However, only one level of melatonin was fed, the period was short and the number of pigs per treatment was minimal. Therefore, the objective of this experiment was to determine the effect of graded concentrations of melatonin on ulcers in growing pigs from 20 kg to market weight.

MATERIALS AND METHODS

Animals and Diets

All animal procedures were approved by the local Animal Care Committee and adhered to the guidelines of the Canadian Council on Animal Care. A total of 64 Yorkshire barrows and gilts were randomly assigned to 16 pens with four pigs per pen. Barrows and gilts were penned separately and litter mates were distributed equally across treatments. Pigs were introduced into the experiment at 22.15 ± 2.04 kg. Pigs were slaughtered after they reached 105 kg and the last pig in the pen was removed when the third pig reached the weight of 105 kg. Individual pig weight and pen feed intake were determined weekly.

The diet was finely ground corn-wheat based diet (Table 1). It was ground with a 2.38-mm screen to achieve a mean particle size of 578 ± 1.89 µm; considered a fine diet with a high probability to induce gastric ulcers. Four levels of melatonin (Sigma Chemical Co., St. Louis, MO) were added to the feed at 0, 2.5, 5, and 10 mg kg⁻¹ of feed. Melatonin was added to the vitamin and mineral premix immediately before feed mixing. Feed was stored in a dark room maintained at approximately 5°C. Feeders were covered to prevent deterioration of melatonin by light.

Postmortem Measurements

On the day of slaughter, pigs remained on feed until transportation to the University of Guelph abattoir and were then slaughtered within 1 h. At slaughter, stomachs were tied off at the oesophageal and pyloric ends to prevent loss of stomach contents. pH readings of the stomach digesta were taken (Fisher Scientific Mini pH Meter, Fisher, Toronto, ON) to determine the relationship between pH value and ulcer severity. Stomach contents were removed, mixed and a 100-g digesta sample was removed and freeze dried to determine total water content. A digesta subsample was centrifuged and analyzed for bile acids using Enzebile (Nycomed, Oslo, Norway), an enzymatic, colorimetric determination of total bile acids. Ulcers of the pars oesophagea were scored after slaughter from 0 to 3; 0 = normal, 1 = parakeratosis, 2 = focal shallow ulceration, 3 = fully developed ulcer.

Statistical Analyses

This study was designed as a randomized complete block design, with treatment, sex, and pen as the experimental unit. All analysis conducted used the General Linear Model

Table 1. Ingredients and analyzed composition of the diet

Item	%
Corn	40.0
Wheat	39.65
Soybean meal, 48% CP	17.0
Limestone	1.0
Dicalcium phosphate	1.5
Cobalt salt	0.50
Mineral premix ^z	0.50
Vitamin premix ^y	0.25
Composition (calculated)	
ME (MJ kg ⁻¹) ^x	13.61
<i>Composition (analyzed)(% DM)^w</i>	
GE (MJ kg ⁻¹)	14.82
Dry matter	87.56
Crude protein	19.19
Ether extract	3.0
Ash	5.67
Crude fibre	2.96
Calcium	1.03
Phosphorus	0.88
Sodium	0.37

^zSupplied per kilogram of diet: copper 15 mg, zinc 100 mg, iron 100 mg, manganese 20 mg, iodine 0.3 mg, selenium 0.3 mg, 0, 2.5, 5 or 10 mg melatonin.

^ySupplied per kilogram diet: 6000 IU vitamin A, 600 IU vitamin D₃, 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, 15 IU vitamin B₁₂.

^xCalculated from published values of diet ingredients from NRC for swine (National Research Council 1988).

^wAnalyzed by methods of Association of Official Analytical Chemists (1990).

(GLM) procedure (SAS, V. 6.06, SAS Institute, Cary, NC). The GLM procedure was used to test the effect of melatonin treatment on average daily gain, feed/gain, feed intake and ulcer scores. Least squares (LS) means were calculated and protected least significant difference (LSD) was performed to compare treatment responses. Data were tested for linear, quadratic, and cubic effects. GLM procedure was performed to test for effect of ulcer score on average daily gain, feed/gain, feed intake, and pH of stomach contents. The data on bile acid concentration and digesta volume were normalized using log transformation and then analyzed using the GLM procedure. Values are reported as antilog of log means. If the *F* value for ulcer effect was significant, differences between LS means were assessed.

RESULTS AND DISCUSSION

This study suggests that melatonin may be related to gastric ulcer development in swine. Pigs that were supplemented with melatonin had significantly fewer ulcers and less severe ulcers than those pigs not supplemented with melatonin (Table 2). These data confirm our previous observation of decreased ulcer prevalence and severity in 22-kg pigs receiving 5 mg melatonin kg⁻¹ diet for 4 wk (Ayles et al. 1995). There was no dose-dependent response; 2.5 mg kg⁻¹ had the same effects as 5 and 10 mg kg⁻¹ on decreasing ulcer severity. Perhaps a lower dose of melatonin may have

Table 2. Effect of graded levels of dietary melatonin on gain, feed intake and feed efficiency in pigs fed a finely ground diet (578 µm) for 14 wk

Dietary melatonin (mg kg ⁻¹)	0	2.5	5	10	SEM	P
<i>n</i>	16	16	16	16		
Initial weight (kg)	23.2	23.1	23.2	21.4	0.37	NS ^z
Final weight (kg)	104.5	102.6	101.3	102.3	0.93	NS
ADG (kg d ⁻¹)	0.92	0.90	0.93	0.92	0.01	NS
Feed intake ^y (kg d ⁻¹)	2.49 ^a	2.51 ^a	2.46 ^{ab}	2.39 ^b	0.05	0.08
Feed/gain ^y (kg kg ⁻¹)	2.60	2.75	2.63	2.68	0.08	NS
Prevalence GU (%) ^x	100 ^a	87.5 ^b	81.5 ^b	81 ^b	0.28	0.05
Mean ulcer score ^{xw}	2.07 ^a	1.56 ^b	1.60 ^b	1.60 ^b	0.12	0.05
Bile acid ^v (µmol/L)	2396 ^a	96 ^b	117 ^b	125 ^b	164	0.05

ⁿ16 pens, four pigs/pen.

^zNS = no significant difference.

^yFour pigs per pen, analysis conducted on a pen basis.

^xLinear effect, $P < 0.05$.

^wUlcer scoring system: 0 = normal, 1 = parakeratosis, 2 = focal shallow ulceration, 3 = fully developed ulcer; linear effect, $P < 0.05$.

^vNormalized by log transformation; means are antilog of log means

a, b Different letters $P < 0.05$.

the same effects as 2.5 mg kg⁻¹, making it more economical for practical use.

Melatonin supplementation significantly ($P < 0.05$) reduced bile acid concentration of stomach contents compared with pigs receiving the control diet (Table 2). High concentration of bile acid in gastric contents have often been found in human patients with gastric ulcers (Stein et al. 1992). The cause and effect relationships are not clear, but deserve further study.

The highest level of melatonin (10 mg kg⁻¹) produced a slight reduction ($P < 0.08$) in feed intake (Table 2). However, there were no differences in growth rates for pigs supplemented with and without melatonin (Table 2). Serotonin administration has been shown to decrease food intake in rats (Pollock and Rowlands 1981), decrease HCl secretions (Ormsbee and Fondacaro 1982) and increase the motility of the gastrointestinal tract (Bubenik 1986). It was postulated by Bubenik and Pang (1994) that under certain circumstances serotonin and melatonin may have parallel effects, particularly in respect to lowering of feed intake since sows were found to have an increase in plasma melatonin at feeding time (Brandt et al. 1986). Deboer (1988) detected a rise in plasma melatonin in sows in the light phase at feeding times. This rise in melatonin at feeding may affect motility of the gastrointestinal tract, by slowing the rate of passage of digesta, allowing for the absorption of more nutrients, and thus increasing feed efficiency. Ayles et al. (1995) found an increase in apparent crude protein and dry matter digestibility of 3.7 ($P < 0.04$) and 3.0 ($P < 0.08$) percentage units, respectively, when 5 mg melatonin was fed to 22-kg pigs.

There was no relationship between ulcer score and percent moisture of stomach digesta (Table 3), contrary to the reports by Mahan et al. (1966), Reimann et al. (1968), Maxwell et al. (1970) and Maxwell et al. (1972). It was reported in these previous studies that pigs with ulcers had a higher percent moisture, with an increase in fluidity. In these earlier studies, pigs were killed and the stomachs were removed immediately (Mahan et al. 1966) or the stomachs of the pigs were fistulated (Reimann et al. 1968; Maxwell et al. 1970, 1972). In the present study, pigs were killed in a

commercial slaughter plant where they had access to water. The fluid nature of the stomach, when a fine diet is fed, is believed to allow more pepsin and HCL to come in contact with the pars oesophageal area. What accounts for the increase in moisture is not clear; it may be due to an increase in gastric secretion when a fine-ground diet is fed, an increase in bile acid reflux from the small intestine or an increase in water consumption prior to slaughter. When a coarse diet is fed, it has been shown that little mixing is occurring and the stomach contents have a lower percent moisture than when a fine diet is fed (Maxwell et al. 1972). In the present experiment, there were no differences in percent moisture in stomach contents, or in total volume of the stomach contents (Table 3).

There was no difference in pH of stomach contents between pigs with ulcers and those without ulcers (Table 3), possibly because the pH value taken at slaughter did not represent the pH value at the time of the development of the ulcer. The pH values in this study do not indicate whether the pH gradient between the pars oesophageal area and the pyloric area was disturbed. The pH readings were taken at slaughter after the animals had been subjected to considerable movement. To observe the possible differences in the pH gradient within the stomach would require anaesthetizing the pig and removing the stomach with little movement involved. Unfortunately, this process reduces or destroys the economic value of the carcass.

Pigs with severe ulcers had higher bile acid concentrations in the stomach than pigs with no ulcers (Table 3). Increased mixing of the stomach contents when a finely ground feed is fed, along with the natural motility of the stomach, may cause a reflux of bile acids from the small intestine. Endoscopic examination of duodenogastric reflux disease in humans revealed that a large bile lake is often observed in the stomach in patients with severe ulceration (Stein et al. 1992). Bile acids could play a major role in the development of gastric ulcers by allowing an excess of H⁺ ions to backflow on the mucus membrane of the pars oesophagea. This appears to be the first report of an association between gastric ulcers and bile acid concentration in the stomach digesta of pigs.

Table 3. Relationship between ulcer score and ADG, percent water of stomach contents, bile acid concentration of the digesta and pH of stomach fluids of pigs² fed a finely ground diet (578 μm) for 14 wk

Ulcer score ³	0	1	2	3	SEM
<i>n</i>	7	23	18	16	
ADG (kg d ⁻¹)	0.94	0.91	0.92	0.92	0.01
% water	66.6	64.6	63.7	66.2	0.95
Bile acid ⁴ ($\mu\text{mol L}^{-1}$)	45 _c	684 _b	613 _b	1785 _a	164
pH	4.38	4.60	4.46	4.69	0.10

n = number of pigs.

²Initial weight 22.15 \pm 2.04 kg, final weight 102.4 \pm 4.3 kg.

³Ulcer scoring system: 0 = normal, 1 = parakeratosis, 2 = focal shallow ulceration, 3 = fully developed ulcer.

⁴Normalized by log transformation; means are antilog of log means

a, b Different letters *P* < 0.05.

Melatonin has been speculated to act by counteracting the effects of serotonin to decrease the occurrence of gastric ulcers (Cho et al. 1989). Although the serotonin and melatonin interaction is probably involved, it is unlikely to be the sole cause of a gastric ulcer. An increase in acid load alone will not cause an ulcer, but an increase in HCl secretion and an increase in gastrointestinal motility, along with a decrease in gastric blood flow, will cause the development of an ulcer (Tsukamoto et al. 1991). The etiology of gastric ulcers is complex and is not yet fully understood, therefore we cannot conclude that serotonin is the sole cause of an ulcer and that with the addition of exogenous melatonin, ulcers will not develop.

This study clearly indicates that melatonin is involved in some manner in gastric ulceration in pigs. Melatonin supplementation significantly decreased the prevalence and severity of ulcers, and decreased feed intake of pigs that were supplemented with 10 mg melatonin kg⁻¹ diet. By decreasing the feed requirements, increasing the apparent digestibility of the diet (Ayles et al. 1995) and decreasing the prevalence and severity of ulcers, melatonin supplementation may have a positive impact on production economics.

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