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The influence of a magnesium rich marine supplement on behaviour, salivary cortisol levels, and skin lesions in growing pigs exposed to acute stressors

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ABSTRACT

Pigs in intensive production systems typically experience multiple acute stressors which can have a negative impact on their welfare. This study investigated whether a magnesium (Mg) rich marine extract (SUPPLEMENT) could reduce the negative effects of mixing and an out-of-feed event on pig welfare. At weaning (28 d) 448 piglets were assigned to either control or SUPPLEMENT (0.5% of the diet) diets in single sex groups of 14. Four weeks later (day 56, c. 17 kg) pigs were blocked according to weight and back-test scores. Seven piglets from each pen were mixed with 7 from another of the same sex and dietary treatment to yield the following groups: control male, SUPPLEMENT male, control female and SUPPLEMENT female ($n = 4$ of each). At mixing, behaviour was recorded on video for 3 h and the frequency and duration of aggressive behaviours, as well as the number of pigs involved in each bout of aggression was recorded. Additionally, the proportion of pigs standing or lying was recorded at 10 min intervals. At 112 d feed was removed for 21 h. After re-introduction of the feed, pens were observed continuously for 8 × 2 min periods and aggressive behaviour was recorded. Skin lesions of 4 focal pigs/pen were scored on the day before and after mixing and the out-of-feed event. Saliva samples were collected on day 56 and day 113 (1 h before and 1, 3 and 8 h after mixing/feed delivery post deprivation) and at 10:00 h on day 55, day 57, day 58, day 112 and day 114 by allowing the 4 focal pigs to chew on a cotton bud for 1 min. Cortisol was analysed by ELISA. At mixing, aggressive interactions between males lasted longer than between females (34:27 vs. 16:55 mm:ss, s.e. 03:38; $P < 0.01$) and more control than SUPPLEMENT pigs were involved in each bout of aggression (2.13 ± 0.39 vs. 2.08 ± 0.34; $P < 0.05$). There were no treatment effects on the frequency of aggressive behaviours ($P > 0.05$). There was no effect of diet or sex on skin lesion scores, but SUPPLEMENT females had lower cortisol concentrations than control females (1.51 ± 0.12 vs. 1.91 ± 0.13 ng/ml; $P < 0.05$). During the out-of-feed event, neither sex nor diet affected salivary cortisol levels, but males were more aggressive than females (0.182 vs. 0.122 aggressive interactions/pig/min; s.e. 0.019; $P < 0.05$), and control pigs had higher skin lesion scores than SUPPLEMENT pigs (13.2 ± 1.1 vs. 10.0 ± 1.0; $P < 0.05$). These findings suggest that the Mg supplement used in this study had some beneficial effects on pig welfare.

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1. Introduction

Growing pigs in modern production systems typically experience multiple stressors during their lifetime, which can have a negative impact on their health, behaviour and welfare. One of the most common of these is the mixing of unfamiliar pigs. Mixing causes a sharp increase in aggression as the animals attempt to establish a dominance hierarchy (O'Connell et al., 2005; Parratt et al., 2006). This leads not only to injury (Turner et al., 2006) but can also initiate activation of the hypothalamic pituitary adrenal axis, leading to an increase in cortisol secretion (Merlot et al., 2004) and reducing performance (Rundgren and Lofquist, 1989). Management practices, such as grouping newly weaned pigs of similar weights together mean that mixing pigs at least once is largely unavoidable. However, remixing is practiced at other stages in the production cycle on many pig units.

There are strategies that can help reduce aggression, or at least its effects, including the application of pheromones (Guy et al., 2009), adjustment of the time of mixing (Ogunbameru et al., 1992), and provision of barriers in the pen for pigs to avoid confrontation (Waran and Broom, 1993; Ishiwata et al., 2002). However, other unexpected stressors, such as out-of-feed events, where pigs are deprived of feed due to human error, delayed delivery, or equipment malfunction, are not as easy to mitigate. Competition for access to feed once feed has been restored can lead to substantial increases in aggression in the pen (Jensen and Pedersen, 2010). Certainly pigs increase their time at the feeder during the first few hours after feed is restored (Beattie et al., 2002; Jensen and Pedersen, 2010). Feed deprivation in itself could also increase stress and cause poor welfare due to feelings of hunger (Lawrence et al., 1988) and can also result in undesirable social behaviours, such as pen-mate manipulation (Robert et al., 1991). Thus management strategies that could ameliorate the impact of an unexpected stressful event such as out of feed events also warrant investigation.

One option is to supplement the pigs' diet with substances that help to calm pigs and reduce aggression and stress. The welfare benefits of supplementing the diet with additional fibre are already well established for sows where 'calming' effects arising from better gut fill and satiety have been reported (e.g. Stewart et al., 2008, 2010). Supplementary tryptophan has a similar effect on grower gilts, leading to lower activity levels, less aggression and more lying (Poletto et al., 2010). Supplementation of pig diets with magnesium (Mg) throughout the growing period reduced salivary cortisol, aggression, some measures of harmful behaviour, skin lesions in female pigs, and mounting behaviour in male pigs (O'Driscoll et al., in press).

With regard to acutely stressful situations, pigs supplemented with Mg had fewer loin lesions after transport, and lay down more than unsupplemented pigs in a vibrating transport simulator (Peeters et al., 2005, 2006). Peeters et al. (2005) suggested that Mg supplementation improved pigs' coping ability by blocking the sympathetic pathways of the autonomic nervous system. A lot of the focus in research has been on the effect of Mg supplementation

Table 1
Typical mineral composition of the mineral rich marine extract used to supplement pigs in this study.

Mineral	Dry salt weight
Calcium	303,400 ppm
Magnesium	59,520 ppm
Phosphorous	69.4 ppm
Potassium	705 ppm
Iron	1556 ppm
Boron	34.7 ppm
Sodium	8456 ppm
Manganese	63.4 ppm
Cobalt	0.37 ppm
Copper	0.675 ppm
Zinc	0.728 ppm
Selenium	0.785 ppm
Silicon	106 ppm

on welfare around slaughter and on meat quality (D'Souza et al., 1999; Apple et al., 2000, 2005; Peeters et al., 2005, 2006), but to our knowledge there have been no studies of its effect on pig welfare during other acutely stressful situations on farm.

The source of dietary Mg influences an animal's ability to absorb and utilise it and hence its efficacy (D'Souza et al., 1999, 2000). Inorganic forms of Mg are often significantly less available to the animal compared to sources that originate from organic sources (Coudray et al., 2005). A mineral-rich marine extract obtained from the skeletal remains of a red marine algae, *Lithothamnion calcareum*, was used in this study. This alga grows in the Atlantic waters off the southwest of Ireland and the northwest coast of Iceland. Minerals from seawater are accumulated in the algae frond, which breaks off and falls to the ocean floor from where they are harvested. The mineralised fronds are separated from extraneous materials, sterilised, dried and milled. A commercial product is derived from this alga, and contains 28% calcium (Ca) and 5% magnesium (Mg) (as well as measureable levels of 72 other trace minerals) and is sold as a dietary supplement for farm animals (Table 1).

This study investigated whether this SUPPLEMENT could improve the welfare of pigs with undocked (intact) tails during two separate stressful events; mixing of unfamiliar pigs, and an out-of-feed event. We hypothesised that pigs fed a diet supplemented with SUPPLEMENT would express fewer harmful (e.g. tail-biting, belly nosing and ear biting) and aggressive behaviours, have lower aggression induced body lesion scores, and have lower salivary cortisol levels compared with unsupplemented pigs in response to these two stressors. During the study levels of other minerals that are present in SUPPLEMENT were controlled, as our hypotheses were based upon the premise that Mg could exert a calming effect on the pigs.

2. Materials and methods

The study was carried out using the Teagasc integrated 250 sow research herd based at Moorepark, Fermoy, Co. Cork, Ireland. All procedures were reviewed and agreed by Teagasc's Animal Ethics Committee.

2.1. Dietary treatments

Animals were offered a standard, pelleted, commercial diet ad libitum (Vigour, Nutec, Naas, Co. Kildare, Ireland) from multi-space feeders during the first week post weaning. From one week post-weaning all pigs were switched to a home compounded, pelleted diet (Table 2) with treatment groups being supplemented with SUPPLEMENT at a rate of 0.5%.

2.2. Animals and housing

A total of 448 undocked entire male and female piglets, born from Large White × Landrace sows were used. At 28 d piglets were weaned, ear tagged and weighed and assigned to single sex first stage weaner pens in groups of 14 pigs ($n = 32$ pens; 1st stage weaner accommodation). Pens measured (1.3 m × 2.8 m). Feed intake at the pen level was recorded daily. Water was available ad libitum from a single bite-drinker in each pen. Each pen was furnished with a metre length of a natural fibre (manila) rope suspended from the pen partition and a length of plastic piping through which a chain was passed and fixed at both ends to the wall to contribute towards the environmental enrichment requirements of the pigs. Second stage weaner accommodation consisted of pens (1.32 m × 3.82 m) with plastic slatted flooring furnished with a Bite Rite™ enrichment device (Ikadan System, Denmark) consisting of a plastic cone with four protruding lengths of plastic (length 20 cm, diameter 1 cm), suspended from the ceiling at pig head level in the middle of the pen. There were also two chains (approx 50 cm) hanging from the pen walls. The finisher accommodation consisted of pens (2.32 m × 4.73 m) with fully slatted, concrete floors and furnished with a 'home made' rubber enrichment device suspended at pig height in the middle of the pen, and two chains hanging from the pen walls.

2.3. Treatments/experimental design

Treatments and experimental design are described in detail by O'Driscoll et al. (in press). In brief, all piglets were subjected to a back-test at 27 d. At weaning pigs were assigned on the basis of sex and weight to one of four experimental treatments: (1) male control; (2) male SUPPLEMENT; (3) female control and (4) female SUPPLEMENT. A balanced incomplete block design was used, with each treatment replicated 4 times. At 56 d pigs were weighed and then re-mixed (09:30 h) into the 2nd stage weaner accommodation. Seven pigs from each 1st stage pen were selected on the basis of their back test score and weight, then mixed with 7 pigs from another pen of

Table 2
Ingredient and chemical composition of the diets fed at each of 3 production stages.

	1st weaner stage		2nd weaner stage		Finisher stage	
	Control	SP[a]	Control	SP[a]	Control	SP[a]
Ingredient						
Barley	0	0	250	250	400	400
Wheat	411	408.8	450	448.7	387.5	386.5
Soya Hi-Pro	82.1	82.4	200	200	175	175
Provisoy	100	100	0	0	0	0
Soya full-fat	100	100	50	50	0	0
Lactofeed 70	200	200	0	0	0	0
Whey dried cheese	50	50	0	0	0	0
Fat, soya oil	31.8	32.6	18.3	18.9	10	10
Lysine HCl	4	4	4.5	4.5	3.9	3.9
DL-Methionine	2.3	2.3	1.6	1.5	0.8	0.9
L-Threonine	1.6	1.6	1.8	1.8	1.3	1.3
L-Tryptophan	0.5	0.5	0	0	0	0
Vit/min premix[b]	3	3	1	1	1	1
Salt	1	1	3.5	3.4	2.9	2.8
Mono DiCal Phos	5.2	5.2	8.3	8.3	4	4
Limestone flour	7.5	3.6	11	6.9	13.6	9.6
Acid-Buf™	0	5	0	5	0	5
Chemical composition						
Dry matter[c]	907	903	892	892	892	897
Crude protein[c]	210	208	200	204	177	174
Crude fibre[c]	21	21	31	29	30	32
Ash[c]	51	51	47	46	44	44
Fat[c]	66	66	51	52	34	34
Lysine[d]	14	14	13	13	11	11
Digestible energy[d]	15	15	14	14	13.56	13.55
Ca[c]	0.55	0.57	0.64	0.64	0.62	0.62
Mg[c]	0.19	0.22	0.17	0.20	0.16	0.18

[a] SP represents a diet supplemented with a commercially available dietary supplement extracted from a mineral-rich marine extract.
[b] Premix provided per kilogram of complete diet: Cu, 15 mg; Fe, 24 mg; Mn, 31 mg; Zn, 80 mg; I, 0.3 mg; Se, 0.2 mg; vitamin A, 2000 IU; vitamin D_3, 500 IU; vitamin E, 40 IU; vitamin K, 4 mg; vitamin B_{12}, 15 mg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; vitamin B_1, 2 mg; and vitamin B_6, 3 mg.
[c] Analysed values.
[d] Calculated values.

the same sex and treatment yielding 16 groups of 14 pigs ($n = 4$/treatment). One high scoring pig and one low scoring pig from each 1st stage weaner accommodation pen was selected as a focal pig, providing 4 focal pigs in each 2nd weaner stage pen. At 92 d pigs were weighed and moved in the same groups to the finisher accommodation. The trial ended when the pigs were about 120 d of age or approximately 60 kg live weight. At 112 d pigs were subjected to an out-of-feed event for 21 h. All feed was removed from the troughs and feed bins at 12:00 h (day 1), and bins were not replenished until 09:00 h the following morning (day 2).

2.4. Measurements

2.4.1. Behaviour

2.4.1.1. Mixing. On transfer to the 2nd stage accommodation behaviour in each pen was recorded continuously for 3 h from 09:30 h using a time lapse video recorder connected to a multiplexer. The number of pigs standing and lying was counted every 10 min during these 3 h ($n = 18$ datapoints/pen). In addition, the video recordings were observed continuously and the number and duration of all aggressive interactions in each pen recorded, as well as the number of pigs involved in each bout of aggression. An aggressive interaction was counted whenever two pigs began interacting aggressively i.e. head knocking, biting and/or levering one another or when one pig directed a single aggressive act (i.e. a head knock, bite or levering) towards another pig which was not reciprocated. When a pig joined an ongoing aggressive interaction this was considered a new bout of aggression. The total duration of aggressive behaviour in the pen was calculated by summing the duration of all aggressive acts. An index of aggression in each pen was calculated by multiplying the duration of each aggressive interaction by the number of pigs that were involved in that interaction.

2.4.1.2. Out-of-feed event. Behaviour observations were carried out by a single observer using direct observation between 09:00 h and 10:00 h and 15:00 h and 16:00 h on day 1 and day 2 of the feed-out event. Each pen was observed continuously for 2 min on 8 occasions during each hour long recording period. On the morning of day 2 the first observation for each pen took place as soon as feed was added to the feed bin. The observer then rotated observations between pens, so that the 8 observation periods for each pen began at approximately 8 min intervals. All incidences of aggressive (head-knocks, bites and fights) and harmful (tail, ear and nose biting, and belly nosing) behaviours were recorded.

2.4.2. Salivary cortisol

Focal pigs were habituated to the saliva collection procedure as described by O'Driscoll et al. (in press). Saliva was collected by allowing pigs to chew on a large cotton bud (Salivette, Sarstedt, Wexford, Ireland) until it was thoroughly moistened (about 30–60 s per sample). The buds were placed in tubes and centrifuged for 10 min at $400 \times g$, then stored at $-20\,^{\circ}$C until analysis by an enzyme linked immunosorbent assay (Salivary Cortisol Kit, Salimetrics Europe Ltd., Suffolk, UK). The minimum detectable concentration of cortisol that could be distinguished from 0 is <0.003 μg/dl. The intra- and inter-assay CVs based on controls were 2.7% and 4.9%, respectively. Saliva samples were collected from focal pigs between 10:00 h and 11:00 h on the day prior to (day − 1) and for 2 d subsequent to mixing (day 1, day 2) and on the first day of, and 1 d subsequent to the feed-out event (day 1, day 3). Saliva was also collected on the day of mixing and on day 2 of the feed-out event between 08:00 h and 09:00 h, 10:00 h and 11:00 h, 12:00 h and 13:00 h and 16:00 h and 17:00 h (i.e. −1, 1, 3 and 8 h after mixing/feed delivery post deprivation). This yielded 7 saliva samples/pig during mixing, and 6 samples/pig during the feed-out event.

2.4.3. Health inspections

2.4.3.1. Tail lesions. The tail of each pig in each pen was examined on the day prior to, and after, mixing (day − 1 and day 1) and the feed out event (day 1 and day 3). Each pen of pigs was removed to an area where pigs could be individually penned and examined. Four parts of the tail were scored according to the system described in Table 3; the distal 1/3, the mid 1/3, the caudal 1/3 and the tail tip. Pigs with fresh wounds to the tail were treated with wound healing ointment (Stalosan® ointment, Stormøllen A/S, Denmark, O'Connor Group, Demesne, Newmarket, Co. Cork, Ireland). If tail damage was severe (loss of part of the tail or deep bite wounds with inflammation) antibiotics were also administered at this time.

Table 3
Tail lesion scoring system.

Score	Name	Description
0	None	Tail intact
1	Red/swelling	Red area, or swelling without redness, appears like most minor injury or recovered area
1	Scratch	1 minor scratch or grazed area, either fresh or scabbed
2	Old scab	Dark colour and dry, no sign of fresh blood
3	Fresh scab	Recent injury, skin broken and blood dried, but not yet fully scabbed
3	Minor bite	Fresh bite mark with blood present, small area, not deep
4	Raw	Top layer of skin removed/inflamed (e.g. from under a scab that has been removed early), but no obvious puncture wound
4	Major bite	Copious blood, severe tissue damage, deep bite
5	Severe wound	Rawness and major bite, with localised inflammation
6	Severe infection	Entire region inflamed, and/or severe wound
7	Amputation	Tail is so badly damaged that it has been removed by other pigs or needs surgical removal

Table 4
Skin lesion scoring system.

Score	Description
0	No lesion
1	1 small
2	>1 small or 1 red
3	>1 red
4	1 deep red
5	>1 deep red or 1 big lesion
6	>1 big lesion

2.4.3.2. Skin lesion scores. Skin lesion scores were recorded for the 4 focal pigs in each pen at the same time as their tails were examined. The back, left and right hind quarters, flanks, head/neck areas and ears were scored according to severity (Table 4). Scores from all areas were summed to provide a total lesion score for each pig. Additionally, scores from the head/neck, ears and shoulders were summed separately as injuries to these areas are predominately caused by agonistic behaviour.

2.5. Statistical analysis

Data were analyzed using the Statistical Analyses System (SAS, version 9.1.3, 1989, SAS Institute Inc., Cary, NC). Data were tested for normality prior to analysis by examination of box and normal distribution plots, and transformed when necessary. The percentage of pigs standing in each pen during the hours after mixing was analysed using the Mixed procedure. Because cortisol was collected approximately 1 h after pigs were mixed, and this required people to enter the pen and disturb the pigs, time points between and inclusive of 70 min and 120 min from mixing were not included in the dataset. Diet, sex, time post mixing, replicate and interactions were inserted into the model as fixed effects. Time was considered a repeated effect. The duration of each individual aggressive interaction during the 3 h recording period was analysed using the Mixed procedure. Diet, sex and replicate were considered fixed effects and the number of pigs involved in each fight was considered a covariate. Fight number was used as a repeated measure. A similar model, but without the repeated effect of fight number, was used to analyse the number of agonistic interactions, the total duration of all agonistic interactions, and the overall time spent in agonistic interactions when considering the number of pigs involved. The non-parametric Kruskall–Wallis test was used to test for the effect of diet and sex on the number of pigs engaged in each agonistic interaction. Behaviour during the feed-out event was analysed using the Mixed procedure. The 8 recordings from each of the four observation periods were averaged to provide one value for each pen during each morning and afternoon observation. Diet, sex, day, time of day, the number of pigs on the pen and replicate were inserted into the model as fixed effects. Time of day, nested within day, was considered a repeated effect.

Salivary cortisol concentration during the mixing and feed-out events was analysed using the Mixed procedure. Diet, sex, day, sample number, whether the pig was a high or low responder to the back test, and replicate were inserted into the models as fixed effects. Elisa plate was considered a random effect, and sample number was considered a repeated effect. Tail lesion scores from each part of each tail were added to provide a total score for each pig on each examination day, then scores in each pen on each examination day were averaged to provide a single value for each pen. Data were analysed using the Mixed procedure. Diet, sex, day replicate were inserted into the model as fixed effects. Day was considered a repeated effect. Total and front of body skin lesion scores during the mixing and feed-out events were analysed using the Mixed procedure. Diet, sex, day, whether the pig was a high or low responder to the back test and replicate were inserted into the model as fixed effects. Day was considered a repeated effect. All mixed models included interaction terms where relevant. When significant effects were found Tukey's test was used to establish pair-wise differences. Statistical differences were considered significant at $P \leq 0.05$. Tendencies towards significance ($0.05 \leq P \leq 0.10$) are also presented. Data are presented as least squares means ± standard errors. Model-fit was determined in all analyses by choosing models with the minimum finite-sample corrected Akaike Information Criteria (AIC).

3. Results

3.1. Mixing

3.1.1. Behaviour

There was a tendency for fewer pigs in the SUPPLEMENT treatment to be standing during the 3 h post mixing, compared with control pigs (49.8 ± 2.6% vs. 51.5 ± 2.6%; $P = 0.08$). There was no effect of sex. The percentage of pigs standing declined over time ($P < 0.001$; Fig. 1). There was no difference between SUPPLEMENT and control pigs in the duration of each individual bout of agonistic behaviour, or in the total duration of agonistic behaviour during the 3 h after mixing. However, the duration in male pens was longer than in female, and the overall duration of agonistic interactions was longer in male than female pens (Table 5). There was no effect of either diet or sex on the number of instances of agonistic interaction. When the duration of agonistic interactions was multiplied by the number of pigs involved in each interaction there was no effect of diet, but

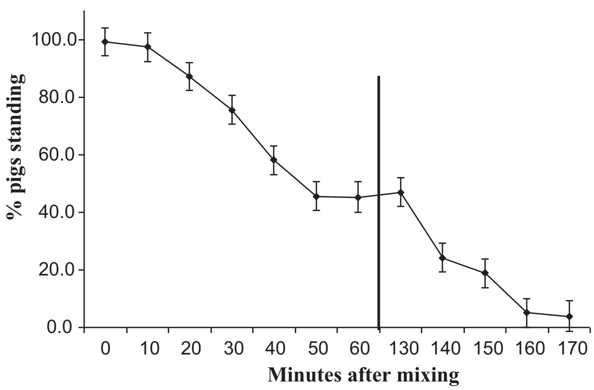

Fig. 1. The percentage of pigs standing at 10 min intervals after mixing. There was a significant effect of time ($P < 0.001$).

Table 5
The effect of sex and supplementation with a magnesium rich marine based supplement on agonistic interactions of growing pigs (age = 56 d) during the 3 h after mixing.

	Sex		Diet		Statistics		
	Female	Male	Supplement	Control	RMSE	Sex (*P*)	Diet (*P*)
Number of bouts	83.0	79.9	88.5	74.4	12.0	NS	NS
Bout duration (mm:ss)	00:12	00:26	00:16	00:23	00:02	0.001	NS
Total duration (mm:ss)	16:55	34:27	23:08	28:14	09:59	0.01	NS
Number of pigs involved/bout[a]	2.08	2.13	2.08	2.13	–	0.05	NS
Bout duration × no. pigs involved (hh:mm:ss)	00:41:42	01:36:16	00:58:30	01:19:28	37:55	0.01	NS

Pigs were observed continuously during this period. The interaction between sex and supplementation was non-significant for all measures. NS = non-significant ($P > 0.05$).
[a] Mean ± standard deviation. For all other measures least squares means are presented.

male pigs spent more time performing agonistic interactions. More control than SUPPLEMENT pigs were involved in each agonistic interaction.

3.1.2. Salivary cortisol

SUPPLEMENT pigs tended to have lower cortisol concentrations (1.67 ± 0.10 ng/ml) than control pigs during the mixing event (1.81 ± 0.10 ng/ml; $P = 0.08$). There was an interaction between sex and diet on cortisol levels ($P < 0.05$). Female SUPPLEMENT pigs had lower cortisol concentrations (1.51 ± 0.12 ng/ml) than female control (1.91 ± 0.13 ng/ml; $P < 0.05$), but there was no difference between control and SUPPLEMENT male pigs (1.70 ± 0.12 ng/ml vs. 1.82 ± 0.13 ng/ml, respectively). The time of sample collection also had an effect on cortisol concentration ($P < 0.05$). Salivary cortisol concentration was higher 1 h after mixing (2.02 ± 0.17 ng/ml) than 8 h after mixing (1.34 ± 0.16 ng/ml; $P < 0.05$).

3.1.3. Health inspections

There was no effect of diet or sex on tail lesion scores during the mixing event, but tail lesion scores tended to be higher on day 2 (day 1 = 1.32 ± 0.40, day 2 = 1.52 ± 0.40; $P = 0.08$). There was no effect of diet or sex on skin lesion scores. However, total skin lesion scores were higher on day 2 (17.0 ± 0.8) than day 1 (5.0 ± 0.8; $P < 0.001$). Neither was there an effect of diet or sex on lesion scores on the front of the body, but again there was an effect of day (day 1 = 2.6 ± 0.5, day 2 = 9.9 ± 0.5; $P < 0.001$).

3.2. Out-of-feed event

3.2.1. Behaviour

There was no effect of diet or sex on total frequency of harmful behaviour, or on tail biting, ear biting, or belly nosing. However, more harmful behaviours were observed on day 1 (0.094 ± 0.005/pig/min) than on day 2 (0.050 ± 0.004/pig/min; $P < 0.001$). Harmful behaviours were observed less in both the morning and afternoon on day 2, than in the morning ($P < 0.001$, $P < 0.01$) or afternoon ($P < 0.001$, $P < 0.01$) on day 1 (Table 6). Pigs performed more tail biting, ear biting and belly nosing on day 1 than day 2 ($P < 0.01$, $P < 0.001$, $P < 0.05$, respectively; Fig. 2). There was no effect of diet on aggressive behaviour. However, male pigs performed more aggressive behaviours (0.182 ± 0.019/pig/min; $P < 0.001$) than female pigs (0.122 ± 0.019/pig/min; $P < 0.05$). There was also an effect of the time of day ($P < 0.001$), and an interaction between time of day, and day; more pigs performed aggressive behaviour on the morning of day 2 than at any other recording period ($P < 0.001$; Table 6). There was no effect of diet or sex on incidence of biting, or of diet on head-knocks. However male pigs performed more head-knocks (0.131 ± 0.014/pig/min) than female pigs (0.078 ± 0.014/pig/min; $P < 0.05$). There was also an interaction between day and time of day for both head-knock and biting behaviour ($P < 0.001$ for both; Table 6). Pigs performed fewer head-knocks during the afternoon on day 2 than in the morning of day 2 ($P < 0.001$), the afternoon of day 1 ($P = 0.01$), and in the morning of day 1 ($P = 0.05$), and more biting behaviour during the morning on day 2 than on the morning of day 1 ($P < 0.01$), the afternoon of day 1 ($P < 0.001$), and the afternoon of day 2 ($P < 0.001$).

3.2.2. Salivary cortisol

There was no effect of sex or diet on salivary cortisol concentrations during the feed-out event. However, there was an effect of recording time ($P < 0.001$), and there tended to be an interaction between sex and recording time (Fig. 3; $P = 0.1$).

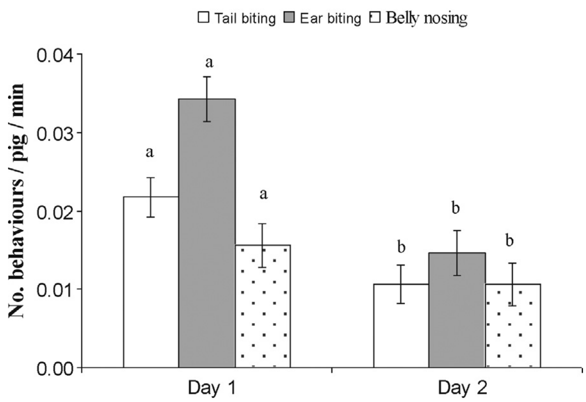

Fig. 2. Instances of tail biting, ear biting, and belly nosing on days 1 and 2 of the out-of-feed event. Behaviour was recorded continuously in each pen for 8 min in the morning (08:00–09:00 h) and afternoon (15:00–16:00 h) of both days, and each instance of each behaviour recorded. Pigs were deprived of feed between 12:00 h on day 1 and 08:00 h on day 2.

Table 6
The number of instances of total harmful, total aggressive, biting and head-knock behaviours per pig per minute in the morning (08:00–09:00) and afternoon (15:00–16:00) of both days of the out-of-feed event.

	Observation time				Statistics			
	Day 1 AM	Day 1 PM	Day 2 AM	Day 2 PM	RMSE	Time (P)	Day (P)	Time × day (P)
All harmful	0.095[a]	0.092[a]	0.041[b]	0.059[b]	0.050	NS	0.001	NS
All aggressive	0.135[a]	0.154[a]	0.225[b]	0.064[c]	0.173	0.001	NS	0.001
Biting	0.028[a]	0.027[a,b]	0.084[c]	0.012[b,d]	0.129	0.001	NS	0.001
Head knocking	0.099[a]	0.121[a]	0.152[a]	0.047[b]	0.056	0.01	NS	0.001

Behaviour was recorded continuously in each pen for 2 min during each period, and each instance of behaviour recorded. Pigs were deprived of feed between 12:00 on day 1 and 08:00 on day 2. NS = non-significant ($P > 0.05$). Differences in superscripts within each behaviour category indicate significant differences.

3.2.3. Health inspections

There was no effect of diet on tail lesion scores during the feed-out event, but female pigs tended to have higher tail lesion scores (2.28 ± 0.72) than male pigs (0.55 ± 0.72; $P < 0.09$), and scores were higher the day after the feed-out event (1.51 ± 0.51) than the day before (1.32 ± 0.51; $P = 0.01$). Moreover, there was an interaction between sex and examination day ($P < 0.05$). In female pigs there was no difference in tail lesion scores the day before (2.29 ± 0.72) or after (2.28 ± 0.72) the feed-out event. However, male pigs had higher tail lesion scores the day after (0.74 ± 0.72) than the day before (0.36 ± 0.72; $P < 0.01$) the out of feed event. Control pigs had higher total lesion scores (13.2 ± 1.1) during the out-of-feed event than SUPPLEMENT pigs (10.0 ± 1.0; $P < 0.05$), and male pigs tended to have higher scores (12.9 ± 1.1) than female pigs (10.3 ± 1.0; $P = 0.07$). Lesion scores were lower on day 1 (10.7 ± 0.9) than day 2 (12.6 ± 0.9; $P < 0.01$), and there tended to be an interaction between diet and day ($P = 0.08$). There was no difference between SUPPLEMENT and control pigs on day 1, but on day 2 control pigs had higher lesion scores than SUPPLEMENT (Fig. 4; $P < 0.05$). Moreover, there was no difference between scores on day 1 and day 2 for SUPPLEMENT pigs, whereas control pigs had higher scores on day 2 than day 1 ($P < 0.01$). Similar to total scores, control pigs had higher lesion scores to the front of the body (7.9 ± 0.6) than SUPPLEMENT pigs (5.6 ± 0.6; $P < 0.01$), and male pigs had higher scores (7.6 ± 0.6) than female pigs (5.9 ± 0.6; $P < 0.05$). Scores were higher on day 2 (7.5 ± 0.5) than day 1 (6.0 ± 0.5; $P < 0.001$).

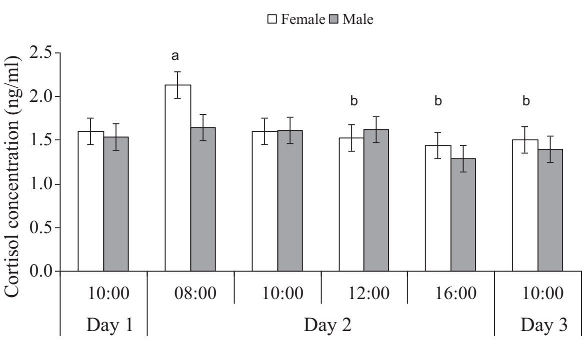

Fig. 3. Salivary cortisol concentration of male and female pigs during an out-of-feed event. Pigs were deprived of feed between 12:00 h on day 1 and 09:00 h on day 2, when feed was added back in to the feed bins. There tended to be an interaction between sex and recording ($P = 0.1$).

4. Discussion

This study used two different types of acutely stressful situations (mixing of unfamiliar animals and feed deprivation) to assess the effect that a dietary supplement containing highly bio-available Mg could have on pig welfare. A multidisciplinary approach to the measurement of welfare was employed in the study, encompassing health, behavioural and physiological indicators. Welfare improvements as a consequence of Mg supplementation were reflected in all indicators; SUPPLEMENT reduced some measures of aggressive behaviour performed after mixing, and tended to reduce salivary cortisol levels. Moreover, lesion scores to the front of the body, and over the entire body, were reduced during an out-of-feed event. However, the improvements were not consistent across both stressful situations, and did not affect both sexes to the same extent.

Unfortunately, because of the necessity of using the pen as the experimental unit for the behavioural measures, there were only 4 experimental units per treatment. Except for the number of agonistic interactions, SUPPLEMENT pigs performed numerically fewer agonistic behaviours than control pigs for all measures during the 3 h after mixing; supplemented pigs had shorter agonistic interactions, spent less time overall in agonistic interactions, and had

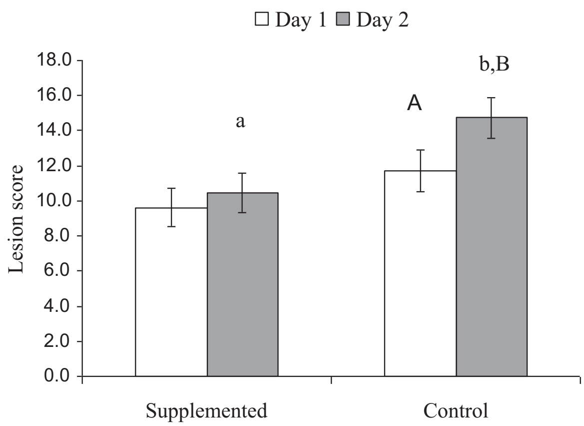

Fig. 4. Lesion scores of pigs on a control diet or supplemented with a magnesium rich marine based supplement on days 1 and 2 of an out-of-feed event. Pigs were deprived of feed between 12:00 h on day 1 and 08:00 h on day 2. a,b indicates a difference between scores of supplement and control pigs on day 2, and A,B indicates a difference between scores on days 1 and 2 for control pigs.

fewer minutes of aggression when the duration for each pig was summed. Thus although there were more agonistic interactions in SUPPLEMENT pens, it appears that these interactions were resolved more quickly than in control pens. These data, in combination with the fact that fewer SUPPLEMENT pigs were involved in each agonistic interaction, imply that the overall level of aggression after mixing was lower for SUPPLEMENT pigs than control. Pigs engage in aggressive behaviour to form a dominance hierarchy, and its establishment is crucial in reducing aggression. It is recognised that the early rearing environment can be instrumental in affecting the formation of a stable dominance hierarchy after mixing, both at weaning and later on in life (Hillmann et al., 2003; Olsson et al., 1999). However little is known about how other management factors could also impact hierarchy formation. The higher number of short aggressive interactions in the SUPPLEMENT treatment immediately post mixing implies that these pigs determined the dominance hierarchy faster than the control pigs (D'Eath, 2005). Thus supplementation with Mg could be a useful tool in reducing aggression at mixing, in situations where manipulation of the rearing environment is not feasible. It is probable that with an increased number of experimental units the differences in duration of behaviour would have been significantly different, and thus further work should be carried out.

Mg supplementation has previously been described as having a quietening effect on pig behaviour (Kuhn et al., 1981; Peeters et al., 2005). It is hypothesised that this is because Mg decreases neuromuscular stimulation (Kietzmann and Jablonski, 1985), due to its antagonistic effect on calcium (D'Souza et al., 1998). Mg supplementation also reduces plasma norepinephrine concentrations and the incidence of PSE meat, which are again indicators of calmness and decreased susceptibility to stress (D'Souza et al., 1998). In the current study, although pigs in both treatments lay down as time progressed after mixing, more SUPPLEMENT pigs lay down sooner than control pigs. Moreover, cortisol levels in SUPPLEMENT pigs tended to be lower than in control pigs during mixing, which concurs with the results of O'Driscoll et al. (in press), who found that pigs that were provided with the same Mg supplement that was used in this study had lower cortisol levels than non-supplemented pigs. Thus our results are in agreement with those of previous studies, which show that Mg supplementation has a calming effect, possibly through the mechanism of a reduced stress response.

Several of the measures taken highlighted gender differences in response to stress and hence differential effects of Mg supplementation depending on the sex of the pig. During the mixing event, female pigs that were supplemented with Mg had lower salivary cortisol levels than control females, whereas there was no difference between supplemented and control male pigs. However, there was a much stronger effect of sex than dietary treatment on aggressive behaviour after mixing, with male pigs having significantly longer agonistic interactions, a higher overall duration of agonistic behaviour, a higher number of pigs involved in each interaction, and more time engaged in aggressive behaviour when considered on a per pig basis, than female pigs. It is thus possible that the higher level of aggression in the pens of male pigs outweighed any potential calming effect that Mg supplementation could have had.

Similar to at mixing, female pigs had a different pattern of cortisol secretion during the out-of-feed event than male pigs. The circadian pattern of female pigs was much more distinct than in males, having a peak at 08:00 h on day 2 that was significantly higher than levels at 12:00 h or 16:00 h, and also higher than at 10:00 h on day 3. However, no such morning peak in cortisol concentrations was seen in the males. Cortisol normally follows a circadian pattern of secretion, and a peak in the morning has previously been observed in pigs (Janssens et al., 1995). However, blunted circadian cortisol rhythms are often recorded in situations of chronic stress in pigs (Janssens et al., 1995), and could indicate reduced welfare (De Jong et al., 2000). The challenges facing male and female pigs may differ somewhat in confinement systems, due to differences in physiology and behaviour. O'Driscoll et al. (in press) found that male pigs had more and longer aggressive interactions during the growing period than females. Another problem in the production of entire male pigs is that they perform sexual behaviour in the form of mounting as pigs mature, which also has negative implications for pig welfare (Cronin et al., 2003). Thus it is possible that male pigs are under more chronic stress than females in intensive production systems. There is also evidence that the response of the HPA-axis to acutely stressful situations is affected by sex (Ruis et al., 1997; Llamas Moya et al., 2006). Thus more detailed sampling of cortisol would be useful to determine if this response is consistent in the lack of an acute stressor, and at what stage circadian cortisol profiles of male and female pigs diverge.

Tail lesion scores increased after both stressful events which is in agreement with the literature. Situations where pigs are stressed or where there is increased activity are hypothesised to trigger tail biting (Schrøder-Petersen and Simonsen, 2001), as well as when there is a breakdown of the social order, such as at mixing (Bracke et al., 2004). The occurrence, severity and outcome of tail-biting is dependant on multiple factors (Taylor et al., 2010). Moreover, 'sudden-forceful' tail-biting, when a pig grabs the tail of another and pulls on it sharply, occurs when pigs have inadequate access to resources so the level of competition is increased (Taylor et al., 2010), which could explain why there was a greater increase in tail lesion scores after the out-of-feed event than after mixing.

As well as an increase in tail lesion scores after the out-of-feed event, there was also an interaction between sex and examination day. Overall, the tail lesion scores of female pigs during the out-of-feed event were higher than male tail lesion scores. Indeed female pigs appear more likely to tail-bite than males (Schrøder-Petersen et al., 2003; Zonderland et al., 2010). However, the females tail scores did not increase during the out of feed event whereas male tail lesion scores showed a significant increase, although they still remained numerically lower than in female pigs. The absence of an increase in tail lesion scores of female pigs after the out-of-feed event is another indication that the acute stress of the out-of-feed event had a more detrimental impact on the male pigs than on the females.

Although there was an effect of sex on tail lesion scores, there was no effect on the occurrence of tail biting behaviour during the mixing event. This may have been because the observation period was not long enough to detect sporadic tail biting behaviour. It may also have been because we recorded all instances of 'tail-in-mouth' behaviour, without recording whether the behaviour was likely to be harmful and lesion-causing, or whether the actor pig simply held the tail in its mouth without chewing (Taylor et al., 2010). More detailed observations could have helped to distinguish between damaging and non-damaging tail-in-mouth behaviour which in turn may have led to better consistency between the tail lesion score and tail biting behaviour data.

During the morning observation period on day 2 of the out-of-feed event aggressive behaviour dominated the pigs behavioural repertoire as they competed for access to the feeders following 21 h of feed deprivation. This explains why lower levels of harmful behaviour were observed at this time. Anecdotally the pigs were lying and resting more later on that day than normally observed; it is likely that they were recuperating after the stress of the out of feed event.

Male pigs spent more time fighting after mixing than females, and performed more aggressive behaviours overall and more head-knock behaviours during the out-of-feed event than female pigs, which is in agreement with previous research (Giersing et al., 2000). Although there were effects of both diet and sex on aggressive behaviour during mixing, these did not translate into differences in skin lesion scores either over the entire body, or for the front portion of the body. However, there was a very large effect of time, with a considerable increase in lesion scores from before to after the mixing event. Mixing and interacting with unfamiliar animals is the main trigger of aggressive behaviour in pigs. It is probable that the resultant duration and severity of agonistic behaviour was high enough that there was no biological difference in the level of injury sustained in either treatment. Of course it is also possible that limitations with our lesion scoring system which focused on the quality rather than on the quantity of skin lesions was not sensitive enough to detect differences between the treatments or sexes.

In contrast while pigs supplemented with Mg had lower skin lesion scores than control pigs during the out of feed event there was no effect of supplementation on aggressive behaviour. This may have been as a result of the limitations of our method of recording behaviour. In contrast to the continuous observations conducted during the mixing event, the necessity of performing direct behaviour observations during the out of feed event meant that the pens could not be observed continuously, indicating that overall these pigs engaged in less aggression. In particular, the interaction between dietary treatment and time implies that supplemented pigs did not express as much aggression after the stress of the out-of-feed event as control pigs, indicating that these pigs may have been better able to cope with this stressor.

5. Conclusions

In conclusion improvements in pig welfare in response to acutely stressful situations as a consequence of Mg supplementation were reflected in all indicators. SUPPLEMENT reduced some measures of aggressive behaviour performed after mixing, and tended to reduce salivary cortisol levels. Moreover, lesion scores to the front of the body, and over the entire body, were reduced during an out-of-feed event. However, the improvements were not consistent across both stressful situations, and did not affect both sexes to the same extent.

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