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Effects of Exogenous Ghrelin on the Behaviors and Performance of Weanling Piglets

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ABSTRACT : The objective of this study was to evaluate the effect of exogenous ghrelin on the behaviors, weight gain, and feed intakes of weanling piglets. A total of 25 pairs of Duroc×Landrace×Large White piglets weaned at 21 days of age were used in this experiment which finished on day 36. Each pair of healthy piglets from the same litter with similar body weight and of the same gender were selected and randomly arranged to ghrelin or control groups. Thus, there were 50 piglets (ghrelin 25; control 25) kept in 10 pens (ghrelin 5; control 5) and 5 piglets per pen. Initial body weight of the pigs did not differ between the control and ghrelin treatment (7.43±0.17 kg; p = 0.81). Experimental pigs were infused with ghrelin (1 μg/d pig) via the marginal ear vein between 0750 and 0800 h at 22, 23, 24 days of age. Control pigs were infused with 0.9% saline. Feed consumption was measured on days 23, 24, 25, 29 and 36. Body weight was measured on days 22, 23, 24, 25, 29 and 36. Behavior data of individual piglets were collected by real-time observation from 0800-1500 h through remote supervisory equipment at 22, 23 and 24 days of age. The results indicated that ghrelin infusion could increase drinking ($p<0.05$) and lying behaviors ($p<0.01$) and decrease mounting behaviors ($p<0.05$). No significant influence of ghrelin was found on average daily weight gain (ADG) and average daily feed intake (ADFI) in this experiment ($p>0.05$). In conclusion, exogenous ghrelin by the method above and at the dosage of 1 μg/d pig could cause a variety of behavioral effects, but not improve performance of weanling piglets. (**Key Words :** Ghrelin, Behavior, Drinking, Piglet, Stress, Weanling)

INTRODUCTION

An acute homeostatic response is provoked by the abruptly social, nutritional, and environmental changes associated with weaning, and this might constitute stress, which piglets typically undergo about 1- to 3-d period of stress-induced, weaning-associated anorexia following (Forbes et al. 1995; Ekkel et al., 1997; Main et al., 2004).

Ghrelin, a novel peptide composed of 28 amino acids found in hypothalamus and stomach, was identified as the endogenous ligand for the growth hormone secretagogue receptor (GHS-R) (Kojima et al., 1999). It was found that both intracerebroventricular (ICV) and intraperitoneal administration of ghrelin in freely feeding rats stimulated food intake (Nakazato et al., 2001).

Salfen (2004) reported that ghrelin could increase weight gain and daily feed intakes throughout the infusion period. Exogenous ghrelin seemed to be a promising solution to the stress of weanling piglets.

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The object of this research was to study the low dosage of exogenous ghrelin on the performance and the behaviors. Behavioral changes were the most consistent and reliable indicators during acute stress (Hicks et al., 1998), we hypothesized that there should be something significantly changed in the weanling piglets with the hormone level fluctuating caused by ghrelin infused.

MATERIALS AND METHODS

Animals, experimental procedure

A total of 25 pairs of Duroc×Landrace×Large White piglets weaned at 21 days of age were used in this experiment which finished on day 36. Each pair of healthy piglets from the same litter with the similar body weight, of the same gender were selected and marked by the same capital or small letter (e.g. A and a) on back and sides with livestock-marking pen, and then randomly arranged to ghrelin or control groups before moved to nursery room (Table 1) in the afternoon of age 21 days. So there were 50 piglets (ghrelin 25; control 25) kept in 10 pens (ghrelin 5; control 5), and 5 piglets per pen. Feed and water were

Table 1. Definition of behaviors

Behaviors	Definitions
Feeding	The head of an animal is over or within feeder for a least 5 s, or the animal is chewing food or grazing, mouth moves in contact with food (Worobec, 1999; Hötze, 2004; Dudink, 2006).
Drinking	Any contact between an animal's mouth and a water nipple (Johnson, 2001; Gardner, 2001).
Fighting	Any behavioral indicatives of social conflict, such as chasing, biting, parallel pressing, head-to-head knocks and levering. The interaction may results in injuries on the body of one or both piglets (Dudink, 2006).
Lying	Piglet's body had contact to the ground and the weight of body is not supported by legs with eyes closed (sleeping) or not (Jeon, 2006; Worobec, 1999; Hötzel, 2004; Hessel, 2006).
Exploratory behavior	The animal is engaged in searching-like activity. Activities involve increased overall alertness, sensory focusing, making nasal contact with parts or objects of the pen or paddock and locomotion accompanied without obvious intention (Hötzel, 2004).
Defecating	Feces be found secreted from anus.
Urinating	Urine found secreted from urethra.
Diarrhea	Frequent and watery bowel movements
Tail-biting	Oral contact with the tail.
Mounting	Put two legs on the other piglet's back.
Belly-nosing	A distinctive sequence in which one piglet rubs a pen-mate's belly with rhythmic, up-and-down movements of its snout (Hötzel, 2004).
Bar-biting	Nosing/chewing parts of the pen (Gardner, 2001).
Gamboling	Bouncing or wallowing with health and vitality, usually one piglet does so first, subsequently the others, even whole piglets within the pen, imitate.

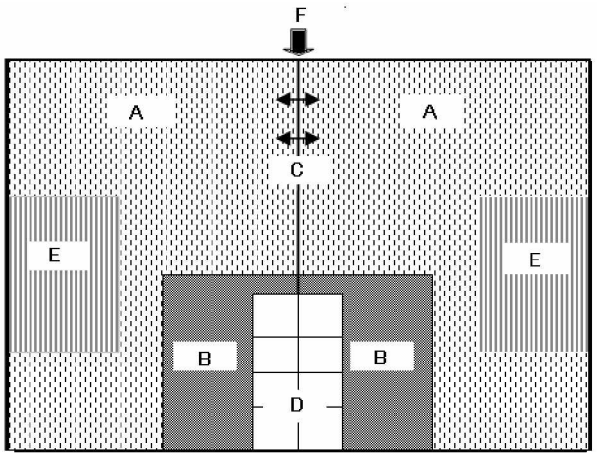

Figure 1. Each nursery pen measured 1.2 m×1.8 m with half slatted (A) and half solid floor (B) contained two nipple drinker (C, flow speed maintained 12 ml/s, water quality according with criterion of human drinking water.) at different height, a four-hole galvanized steel feeders (D), and a board (E) protecting piglets from evil wind under the slatted floor. "F" was the viewing angle of the camera.

offered for *ad libitum* consumption. A single diet was fed to all the piglets during the experiment period.

All the piglets had their tails docked and teeth clipped, and the male had been castrated at birth. To eliminate the adverse effect of changing feed when weaning, the creep feed was used from the day 7 to 36, which meant the same feed was used during the pre-weaning and experiment period. The air temperature 20 cm above the floor in the pen was 20±2°C. Lighting accorded with natural photoperiod.

Ghrelin was diluted 1 μg/ml in physiological saline every 2 d during the infusion period and kept in light-shield glass bottles at 4°C. Experimental pigs were infused ghrelin solution 5 ml/d pig (No. 031-49; Phoenix Pharmaceuticals Inc.) via marginal ear vein between 0750 and 0800 on days 22, 23, 24 after birth, by skillful veterinarians. Control pigs were infused 0.9% saline.

The China Agricultural University Animal Care Committee approved all experimental procedures and all efforts were made to minimize animal suffering due to experimental procedures.

Behavioral observation

One skillful observer made all the behavioral observation. Two pens (one ghrelin, one control piglets) of piglets' behaviors had been record in the special designed tables each time. The observer had not been told which pen control or ghrelin piglets were. The same course was repeated five times to finish the ten pens piglets' behavior record. Behavioral data on the individual piglet were collected. Piglets were identified by the marks on the back and sides. Sampling of each piglet's behavioral data were taken by real-time observation from 0800-1500 at 22, 23, 24 days of age through remote supervisory equipments (CDC-770E, produced by TCL Company). The feeding, lying and exploratory behaviors were record by 3-min intervals, and others behavior were sampled continuously. Cameras were positioned on the wall beside the pen (Figure

1). Any disturbance to the piglets was prohibited during the 7-h observation. All behaviors were allocated to the behavioral categories (Table 1).

Body gain and feed intake

Feed consumption was measured on days 23, 24, 25, 29 and 36. Body weight was measured on days 22, 23, 24, 25, 29 and 36.

Statistical analysis

All the statistics was performed using SPSS 10.0 for windows software (SPSS Inc., 1999). The pen of piglets' behaviors, ADG, and ADFI were regarded as a statistic unit. Normoral distribution was test with Kolmogorov-Smirnov of Nonparametric Tests. If variables of behavior were Normal distribution the Paired-Samples T Test was used to determine changes, and the pens whose piglets from the same litter were regarded as a paired sample, or Two-Independent-Sample Test of Nonparametric Tests were used. To study the relationship among behaviors and ghrelin infusion variables of behavior were analyzed by Factor Analysis of Data Reduction with the method of Principal Components Analysis, and eigenvalue was over 1, maximum iterations for convergence were 25. KMO and Bartlett's Test of Sphericity was adopted to estimate the suitability of the data processed by the method of Principal

Table 2. The influence of ghrelin on the behaviors of early-weaned piglets

Behaviors	Statistics			Paired differences		
	Groups	Mean	SEM	Mean	SEM	Sig.
Feeding	Ghrelin	34.8	2.28	1.86	1.78	NS
	Control	32.9	3.16			
Drinking	Ghrelin	11.3	1.55	2.4	1.1	**
	Control	8.85	0.99			
Fighting	Ghrelin	1.67	0.38	-0.6	0.63	NS
	Control	2.27	0.65			
Lying	Ghrelin	83.6	4.09	5.5	2.17	**
	Control	78.1	4.48			
Exploratory	Ghrelin	22.9	2.54	-3.7	2.05	*
	Control	26.6	2.46			
Defecating	Ghrelin	1.5	0.14	-0.4	0.26	NS
	Control	1.89	0.23			
Urinating	Ghrelin	0.98	0.15	-0.1	0.18	NS
	Control	1.09	0.13			
Mounting	Ghrelin	0.58	0.17	-0.6	1	**
	Control	1.19	0.33			
Diarrhea	Ghrelin	0.12	0.08			NS
	Control	0.13	0.08			
Tail-biting	Ghrelin	0.22	0.1			NS
	Control	0.08	0.04			
Belly-nosing	Ghrelin	0.23	0.08			NS
	Control	0.48	0.2			

Paired-samples t test was used to determine changes of feeding, drinking, fighting, lying, exploratory, defecating, urinating, and mounting behaviors. Two-independent-sample test of nonparametric tests was used to determine the changes of diarrhea, tail-biting, and belly-nosing behaviors for data of them were poisson distributed.

NS = Non significant. The asterisks ** means $p<0.05$; * $p<0.10$.

Table 3. Correlation matrix of ghrelin concentration and behaviors

Correlation	Feeding	Drinking	Fighting	Lying	Exploratory	Defecating	Urinating	Diarrhea	Tail-biting	Mounting	Belly-nosing	Bar-biting	G C
Drinking	0.682***												
Fighting	-0.206	-0.02											
Lying	-0.694***	-0.438***	0.342**										
Exploratory	0.459***	0.232	-0.232	-0.696***									
Defecating	0.12	0.112	-0.031	-0.322**	0.278*								
Urinating	0.025	0.12	0.16	0.068	-0.263*	0.046							
Diarrhea	0.256*	0.01	0.158	-0.234	0.095	-0.003	0.163						
Tail-biting	0.031	0.007	0.267*	0.23	-0.215	-0.089	0.217	0.039					
Mounting	0.710***	0.351**	-0.32**	-0.789***	0.642***	0.289*	-0.058	0.098	-0.219				
Belly-nosing	0.470***	0.189	-0.136	-0.487***	0.326**	-0.131	0.258*	0.354	0.154	0.601***			
Bar-biting	0.346**	0.547***	0.478***	-0.284*	0.186	0.134	0.404**	0.221	0.299*	0.285*	0.438***		
Gamboling	0.174	0.308**	0.228	-0.236	0.096	0.219	0.121	0.078	0.17	0.199	-0.104	0.435***	
G.C.	0.9	0.239	-0.148	0.169	-0.193	-0.267*	-0.092	-0.013	0.239	-0.298*	-0.216	-0.174	-0.119

G.C. = Ghrelin concentration of infusing. The asterisks *** means $p<0.01$; ** $p<0.05$; * $p<0.10$.

Components. Differences were considered great significant at $p<0.01$, significant at $p<0.05$, whereas $p<0.10$ were considered a trend.

RESULTS

Behaviors

The result of all the behavioral variables by One-Sample Kolmogorov-Smirnov Test implied that diarrhea, tail-biting and belly-nosing were Poisson distributed, and no difference was found between ghrlein and control piglets. The others were Normal distributed. The ghrelin pigs had more drinking ($p<0.05$), less mounting ($p<0.05$), and more lying ($p<0.05$) behaviors than control pigs. The control pigs tended to show more exploratory behavior ($p<0.10$) than ghrelin ones (Table 2).

Table 3 indicated that there tended to be negative correlation between ghrelin infusion and defecating ($p<0.10$), ghrelin infusion and mounting behaviors ($p< 0.10$). But significant correlation between ghrelin infusion and feeding behavior was not found ($p>0.10$) despite the correlation coefficient was as high as 0.9. Complicated relationship between behaviors had been represented in the correlation table, e.g. there was great significant positive correlation between feeding and drinking ($p<0.01$), feeding and exploratory ($p<0.01$), feeding and belly-nosing ($p< 0.01$), feeding and bar-biting ($p<0.01$), great significant negative correlation between feeding and lying behaviors ($p<0.01$).

KMO and Barlett's Test's Kaiser-Meyer-Olkin Measure of Sampling Adequacy value was 0.50, Bartlett's Test of Sphericity value was 202.97 ($p<0.01$). There were four principal components to be extracted as showed in Component Score Coefficient Matrix (Table 4), they provided some information of the relationship among the behaviors.

Component 1 reflected the activity of piglets for the coefficients of the mounting (0.21), feeding (0.197), exploratory (0.167), lying (-0.212) were the largest, which could decide the value of component 1. We can see the lying behaviors often reduce the other three behaviors.

Component 2 reflected the interaction among the piglets and between the piglets and environment for the coefficients of the bar-biting (0.308), fighting (0.297), tail-biting (0.260) behavior were the largest. Oral-nasal behaviors against penmates and pen facilities were the common characteristic of these kinds of behaviors.

Component 3 could be defined as an index nutritional for the coefficients of the ghrelin concentration infusion (0.493), feeding (0.226), drinking (0.205) and defecating (-0.37) behaviors were the greatest, which reflected that feeding and drinking behaviors would be increased for the infusion of ghrelin.

Component 4 should be look upon as an index reflected the weanling piglets' adaptability after the infusion of ghrelin, which included some adaptive behaviors such as gamboling (0.335), drinking (0.351), and some maladaptive behaviors such as diarrhea (-0.308) and belly-nosing (-0.439). The coefficient of ghrelin concentration in Component 4 was 0.285, this implied that ghrelin infusion incline to improving the adaptive behaviors.

Average daily weight gain (ADG) and average daily feed intake (ADFI)

Initial body weight of the pigs did not differ between the control and ghrelin treatment groups (7.43±0.17 kg, respectively; $p = 0.81$). There were no differences in ADG or ADFI on postnatal days 22, 23, 24, 25-28, 29-35 between ghrelin and control piglets ($p>0.05$), which suggested that ghrelin administrated with the method above and at the dosage of 1 μg/d pig) could not improve performance of piglets weaned at 21days of age (Tables 5 and 6).

Table 4. Component score coefficient matrix

Behaviors	Component			
	1	2	3	4
Feeding	0.197	0.006	0.226	0.092
Drinking	0.144	0.11	0.205	0.351
Fighting	-0.047	0.297	-0.21	0.013
Lying	-0.212	0.102	0.013	-0.016
Exploratory	0.167	-0.151	-0.091	0.019
Defecating	0.077	-0.032	-0.37	0.213
Urinating	0.018	0.254	-0.009	-0.195
Diarrhea	0.069	0.114	0.056	-0.308
Tail-biting	-0.023	0.26	0.18	0.013
Mounting	0.21	-0.104	-0.047	-0.063
Belly-nosing	0.151	0.063	0.153	-0.439
Bar-biting	0.128	0.308	-0.073	0.023
Gamboling	0.076	0.179	-0.211	0.335
G.C.	-0.051	0.013	0.493	0.285

Extraction method: Principal component analysis. G.C. = Ghrelin concentration of infusing.

Table 5. Effect of intravenous administration of 1 μg/d pig ghrelin or 0.9% saline on average daily weight gain (kg)

Days	Statistics			Paired Differences		
	Groups	Mean	SEM	Mean	SEM	Sig.
22	Ghrelin	0.008	0.029	0.03	0.017	NS
	Control	-0.020	0.023			
23	Ghrelin	0.118	0.034	0.04	0.019	NS
	Control	0.076	0.022			
24	Ghrelin	0.116	0.029	0.00	0.035	NS
	Control	0.120	0.043			
25-28	Ghrelin	0.157	0.026	0.02	0.013	NS
	Control	0.139	0.033			
29-35	Ghrelin	0.441	0.038	0.01	0.017	NS
	Control	0.428	0.026			

Paired-samples t test was used to determine changes. NS = Non significant. The asterisks ** means $p<0.05$; * $p<0.10$.

Table 6. Effect of intravenous administration of 1 μg/d pig ghrelin or 0.9% saline on average daily feed intake (kg)

Days	Statistics			Paired differences		
	Groups	Mean	SEM	Mean	SEM	Sig.
22	Ghrelin	0.075	0.019	-0.011	0.022	NS
	Control	0.086	0.036			
23	Ghrelin	0.158	0.057	0.010	0.045	NS
	Control	0.168	0.075			
24	Ghrelin	0.190	0.044	0.041	0.036	NS
	Control	0.149	0.034			
25-28	Ghrelin	0.223	0.035	0.019	0.022	NS
	Control	0.203	0.048			
29-35	Ghrelin	0.606	0.043	0.006	0.030	NS
	Control	0.600	0.021			

Paired-samples t test was used to determine changes. NS = Non significant. The asterisks ** means $p<0.05$; * $p<0.10$.

DISCUSSION

We were trying to record behaviors in details, so thirteen behavior indexes including feeding, drinking, fighting, lying, exploratory, defecating, urinating, diarrhea, tail-biting, mounting, belly-nosing, bar-biting and gamboling had been adopted to describe a majority of behavioral characters. Feeding, drinking, defecating, urinating were nutrition related behaviors; lying, exploratory behaviors reflected the piglets activity degree (Mateo et al., 2006); some maladaptive behaviors like diarrhea, tail-biting, belly-nosing, and bar-biting often had been regarded as high-incidence behavior in weaning phase (Haskell et al., 1996). Mounting and fighting were interacting behaviors with others piglets, and fighting was the extremely agonistic behavior with subsequent increased injuries unlike some mock combat (pushing, nudging, sudden leaping) and social manipulative (tail-biting, belly-nosing, mounting) behaviors (Glatz, 2001). Gamboling should have been grouped into playing behavior reflecting the activity of animals. Play has been shown to be sensitive to adverse physical and environmental conditions and it has been proposed that play is, thus, an indicator of animal welfare (Tammy et al., 2002). All of the behaviors above could reflect most of the weanling piglets' behavioral characters.

Porcine ghrelin (No. 031-49; Phoenix Pharmaceuticals Inc.) was utilized in this experiment whose amino acid sequence is Gly-Ser-Ser (O-n-ctanoyl)-Phe-Leu-Ser-Pro-Glu-His-Gln-Lys. The apparent half-life of ghrelin following the initial infusion is approximately 15 min in pigs of this age as evidenced by the disappearance rate, and even with this relatively short half-life, serum ghrelin remained above control concentrations throughout 120 min after infusion, during the 8 h after infusion ghrelin was sufficiently cleared (Salfen et al., 2004), therefore, there was no accumulation of ghrelin or other influence related with ghrelin in the serum throughout the experimental time period in this study. Because the activity of ghrelin in the weanling piglets (031-49; 11 amino acid) was unknown, moreover we needed not ensure an elevated serum ghrelin concentration throughout the period of the experiment for the behaviors was only observed from 0800 to 1500, and it was over 17 h interval between two infusions which was long enough to clear up the effects caused by ghrelin, so we attempted to infusion once a day just before 0800 at dosage of 1 μg/d pig.

Usually creep feed was not used after weanling, but changing feed would have great influence on animals, so the creep feed was still used in experiment period (day 22 to 36). Creep feeding of piglets plays an important role in reducing growth check during the adaptation to early

weaning. Most piglets consume less creep feed before weaning (Fraser et al., 1993) and they are initially unfamiliar with the starter diet represented at weaning, resulting in limited consumption of the starter diet and a growth check immediately after weaning. Ghrelin can stimulate gastrointestinal motility and has orexigenic effects (Tolle et al., 2002). However the results indicated that drinking behavior but ADG and feeding had not been significantly changed, this because ghrelin piglets did not get used to solid food even if given a good appetite by infusing ghrelin. Piglets had to drink too much water in order to alleviate hunger. Another reason was that the infusion of exogenous ghrelin could cause hormone level fluctuating greatly, such as increased serum ghrelin GH, insulin, and cortisol concentrations (Salfen et al., 2004), but the relationship between ghrelin and water metabolism had not been made clear, it deserved to be studied further.

Play can be described as behavior that lacks apparent, external goals, `functions', or other obvious goal-directed behavior, it was very difficult to distinguished play from exploratory behavior, they both represent continuous activity, so had been recorded as the same kind of behavior. Ghrelin piglets spent much more time on lying and less time on exploratory behaviors than control ones so we thought ghrelin might have some sedative effect which was in favor of piglets' refreshment from stress.

Though we found ghrelin could decrease mounting behavior of piglets significantly, the causation of mounting behavior in piglets had not been clearly understood. Obviously, it was independent of direct sexual behavior, it happened between the same or different gender.

No significant effects on piglets' behavior of fighting, defecating, urinating, diarrhea, tail-biting and belly-nosing had been found by the treat of infusing ghrelin in this experiment. Fighting decreased the efficiency of food conversion and reduced weight gain (Tan et al., 1991). Presenting food enrichment did not affect the occurrence of aggression or social manipulation (Dudink et al., 2006), for dominance hierarchy re-establishment was inevitable which often initiated within 30 min of mixing piglets, minimizing fighting between piglets may help alleviate some of the stress of the weaning process (Olesen et al., 1996; D` Eath, 2002).

Belly-nosing is characterized by a repetitive rooting motion on the belly of another piglet, similar to the motor pattern used when massaging the sow's udder. Piglets weaned at 7 or 14 days of age spent more time belly-nosing (Worobec et al., 1999), so belly-nosing was referred to as a behavioral indicator of 'stress' in early weaned piglets. Grdner (2001) did not think it to be a general behavioral indicator of stress. But it was consensus that belly-nosing has associated with hunger or feeding motivation (Gonyou et al., 1998; Worobec et al., 1999; Gardner et al., 2001).

In conclusion exogenous ghrelin with the method above and at the dosage of 1 μg/d pig) could caused a variety of behavioral effects, but not improve performance of weanling piglets.

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