Open Access
Asian-Australas J Anim Sci
Vol. 30, No. 12:1796-1802 December 2017
https://doi.org/10.5713/ajas.17.0076
pISSN 1011-2367 eISSN 1976-5517

AJAS
Asian-Australasian Journal of Animal Sciences

Effects of different space allowances on growth performance, blood profile and pork quality in a grow-to-finish production system

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Submitted Feb 13, 2017; Revised Apr 25, 2017; Accepted May 24, 2017

Objective: This experiment was conducted to evaluate the optimal space allowance on growth performance, blood profile and pork quality of growing-finishing pigs.
Methods: A total of ninety crossbred pigs [(Yorkshire×Landrace)×Duroc, 30.25±1.13 kg] were allocated into three treatments (0.96: four pigs/pen, 0.96 m^2/pig; 0.80: five pigs/pen, 0.80 m^2/pig; 0.69: six pigs/pen, 0.69 m^2/pig) in a randomized complete block design. Pigs were housed in balanced sex and had free access to feed in all phases for 14 weeks (growing phase I, growing phase II, finishing phase I, and finishing phase II).
Results: There was no statistical difference in growing phase, but a linear decrease was observed on average daily gain (ADG, $p<0.01$), average daily feed intake (ADFI, $p<0.01$), and body weight (BW, $p<0.01$) with decreasing space allowance in late finishing phase. On the other hand, a quadratic effect was observed on gain to feed ratio in early finishing phase ($p<0.03$). Consequently, overall ADG, ADFI, and final BW linearly declined in response to decreased space allowance ($p<0.01$). The pH of pork had no significant difference in 1 hour after slaughter, whereas there was a linear decrease in 24 h after slaughter with decreasing space allowance. Floor area allowance did not affect pork colors, but shear force linearly increased as floor space decreased ($p<0.01$). There was a linear increase in serum cortisol concentration on 14 week ($p<0.05$) with decreased space allocation. Serum IgG was linearly ameliorated as space allowance increased on 10 week ($p<0.05$) and 14 week ($p<0.01$).
Conclusion: Data from current study indicated that stress derived from reduced space allowance deteriorates the immune system as well as growth performance of pigs, resulting in poor pork quality. Recommended adequate space allowance in a grow-to-finish production system is more than 0.80 m^2/pig for maximizing growth performance and production efficiency.

Keywords: Space Allowance; Growing-finishing Pigs; Growth Performance; Immune; Pork Quality

INTRODUCTION

Large-scale intensive pig farming system has been rising globally due to increase of market demands for pork products during the last two decades [1]. As the public interest in animal welfare increases to the livestock animals, pork producers are confronted with both profitability and welfare issues, although these seem inversely related [2].

Operation of intensive pig production causes various problems, such as growth disturbance, immune dysfunction, risk of exposure to respiratory disease and pork quality deterioration [3,4]. Several studies have been conducted to establish the appropriate space allowance for pigs. NRC [5] recommended the minimum space for maximum ME intake as reported by [6]. The European Union (EU) also established space requirements which were mandated by law (Council Directive 2001/88/EC). Korean Government also legislated space requirements with 0.45 m^2/pig in growing phase (30 to 60 kg) and 0.8 m^2/pig in finishing phase (>80 kg). However, this regulation is only applicable in traditional three stage management systems (weaning, growing, and finishing barn), not the grow-to-finish production systems which are advocated largely because of the ease of management in large-scale farming. However, there is little scientific data available to evaluate the effects of different space allowance on growth performance and pork quality in a grow-to-finish production system.

Therefore, this study evaluated the effect of different space allowance in growing-finishing pigs housed in grow-to-finish production system on productivity as well as economic efficiency.

MATERIALS AND METHODS

Animal care

This experimental protocol was approved by the Ethical Committee for Institutional Animal Use and Care of the Seoul National University (SNU-160613-10). The experiment was conducted at the facility of Seoul National University farm located in Suwon-si, Gyeonggi-do, Republic of Korea.

Animals, experimental designs, diets and housing

A total of 90 crossbred ([Yorkshire×Landrace]×Duroc) pigs, averaging 30.30±1.13 kg initial body weight (BW), were randomly allocated based on initial BW and sex according to randomized complete block (RCB) design with six replicates. Pen size was 1.60×3.00 m, with space allocation achieved by varying the number of pigs per pen. Treatments were i) 0.96 (0.96 m^2/pig, 4 pigs/pen); ii) 0.80 (0.80 m^2/pig, 5 pigs/pen); and iii) 0.69 (0.69 m^2/pig, 6 pigs/pen). A corn-soybean meal based commercial feed was used for 3 phases, including growing (0 to 6 weeks), early finishing (7 to 10 weeks) and late finishing period (10 to 14 weeks). Calculated nutrient contents of the experimental diets are presented in Table 1. Floors were partially slatted, and a climate computer regulated ventilation and heating in the compartments. Temperatures varied between 15°C and 20°C. Lighting was provided in combination with a several windows and fluorescent lights. Each pen had one nipple drinker and feeder. Animals were fed diet and water *ad libitum* during the entire experimental period. The BW and feed consumption were recorded at initial, 3, 6, 10, and 14 weeks to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G/F ratio). Pigs that were removed were weighed and the feed intake and G:F ratio was adjusted based on a model to estimate individual feed intake [7].

Table 1. Calculated nutrient contents of experimental diets

Chemical composition	Growing phase (0 to 6 week)	Early finishing phase (7 to 10 week)	Late finishing phase (11 to 14 week)
ME (kcal/kg)	3,650.00	3,650.00	3,650.00
Crude protein (%)	18.50	15.50	14.00
Crude fat (%)	4.00	4.00	4.00
Crude ash (%)	8.00	8.00	8.00
Lysine (%)	1.10	0.70	0.65
Ca (%)	0.60	0.40	0.35
P (%)	1.30	0.80	0.75

ME, metabolizable energy.

Sampling and measurements

Six randomly selected pigs in each treatment were sacrificed for blood sampling. A 10 mL blood sample from each individual, taken by jugular vein at the same time of measuring BW, was collected in a disposable vacutainer tube without anticoagulant (BD Vacutainer K2E, Becton Dickinson, Plymouth, UK). Then, samples were centrifuged at 3,000 rpm at 4°C for 15 min (Eppendorf centrifuge 5810R, Hamburg, Germany) to separate serum. Samples were stored at –20°C and analyzed for determination of blood urea nitrogen (BUN), cortisol, immunoglobulin A (IgA), and G (IgG). Total BUN concentration was analyzed using a blood analyzer (Ciba-Corning model, Express Plus, Ciba Corning Diagnostics Co., Irvine, MA, USA). For cortisol analysis, samples were analyzed in duplicate within a single assay. Cortisol concentrations were measured using a Coat-a-Count assay kit (Diagnostic Products, Los Angeles, CA, USA). For immunological parameters, serum IgG and IgA of pigs were determined by enzyme-linked immunosorbent assay assay according to the manufacture's protocols (Bethyl Laboratories Inc., Montogomery, TX, USA). The assay was analyzed in duplicate on each serum sample. The assay dynamic range of IgA and IgG were both 15.6 to 10,000 ng/mL.

Pork quality

At the end of experiment, six pigs from each treatment were randomly selected and slaughtered at average 115.98±0.84 kg for the carcass analysis. Pork samples were collected from nearby 10th rib on right side of carcass. After chilling, 1 hour after slaughter was regarded as the initial time. The proximal loin meat was analyzed for dry matter, crude protein, crude fat, and crude ash according to the method of the Association of Official Analytical Chemists [8]. Pork pH and color of longissimus muscle were measured 2 times, 1 h and 24 h after slaughter, respectively. The pH was measured using a pH meter (Φ 500 Series, Bechman Coulter, S. Kraember Blvd Brea, CA, USA) and pork color was measured by Commission Internationale de l'Eclairage color L*, a*, and b* values using a chroma meter (CR-300, Konica Minolta Co., Osaka, Japan). Water holding capacity of pork was measured by centrifuge method [9]. To calculate the cooking loss, longissimus muscles were packed in a polyethylene bag and heated in water bath until core temperature reached 72°C. Weight difference, before and after heating, was regarded as cooking loss. For shear force analysis, samples are cored (0.5×1.0×1.5 cm) parallel to muscle fiber and the cores were used to measure the shear force using a tabletop Warner-Bratzler shear force machine (Saltner Brecknell, Model 235 6X: Motor for Shearer: Bodine Electric Company, Small Motor S/N 0291KUIL 0009 Chicago, IL, USA).

Mortality and economic analysis

A total of 12 pigs were excluded from the experiment (Table 6). Data correction for the ADFI for replication with excluded pigs was adjusted considering the maintenance energy and growth energy of the excluded pigs followed by Kim and Lindemann et al [7]. Pen size was not corrected in the event of pig death or removal. For economic analysis, the days to market weight (115 kg) was calculated from the final body weight and overall ADG (0 to 14 week).

Statistical analysis

Analyses of variance as a RCB design were conducted using PDIFF option with General Linear Model procedures of SAS 9.3 (SAS Inst., Inc., Cary, NC, USA). The pen of pigs was the experimental unit for growth performance (BW, ADG, ADFI, and G/F ratio), and individual pig was used as the experimental unit in hematological analysis and pork quality evaluation. The effects of increasing levels of space allowance were analyzed as linear and quadratic components by orthogonal polynomial contrasts. Pig removal did not conform to the normal distribution and, consequently, the Kruskal-Wallis rank-based nonparametric test [10] was performed using the PROC RANK procedures of SAS. Statistical differences were considered significant at the level of $p<0.05$ and highly significant at the level of $p<0.01$, with a trend between $p\geq 0.05$ and $p\leq 0.10$.

RESULTS

Growth performance

The effect of different space allowance on growth performance is presented in Table 2. There were no significant differences in BW, ADG, ADFI, and G/F ratio in growing phase (0 to 6 week). However, BW was linearly increased ($p<0.01$) as space allowance increased in both early (10 week) and late (14 week) finishing phase, respectively. In addition, there were linear increases in ADG ($p<0.01$) and ADFI ($p<0.01$) as space allowance increased from 0.69 to 0.96 m^2/pig in finishing periods and over the entire experimental period. On the other hand, quadratic response was

Table 2. Effect of different space allowance on growth performance in growing-finishing pigs

Criteria	Treatment[1)]			SEM	p-value	
	0.96	0.80	0.69		Linear	Quadratic
Body weight (kg)						
Initial	30.14	30.43	30.18	1.23	0.74	0.64
3 wk	43.78	42.95	42.05	1.79	0.17	0.97
6 wk	60.70	59.82	59.51	2.10	0.24	0.72
10 wk	80.15	76.08	73.89	2.05	0.01	0.48
14 wk	103.94	98.88	91.40	2.79	0.01	0.64
ADG (g)						
0-3 wk	650	596	565	30.73	0.13	0.80
3-6 wk	806	790	831	23.42	0.60	0.51
6-10 wk	695	581	514	27.53	0.01	0.45
10-14 wk	850	814	625	37.95	0.01	0.14
0-6 wk (Growing)	728	700	698	21.80	0.17	0.45
6-14 wk (Finishing)	772	697	569	28.23	0.01	0.47
Overall	753	698	625	20.32	0.01	0.71
ADFI (g)						
0-3 wk	1,524	1,408	1,446	59.86	0.38	0.32
3-6 wk	2,082	1,919	1,966	59.77	0.30	0.27
6-10 wk	2,216	1,885	1,722	69.20	0.01	0.22
10-14 wk	2,575	2,275	2,116	73.88	0.01	0.42
0-6 wk (Growing)	1,803	1,663	1,706	57.74	0.27	0.23
6-14 wk (Finishing)	2,398	2,072	1,919	68.60	0.01	0.22
Overall	2,143	1,876	1,828	55.90	0.01	0.09
G/F ratio						
0-3 wk	0.426	0.424	0.391	0.011	0.28	0.52
3-6 wk	0.387	0.412	0.423	0.010	0.16	0.74
6-10 wk	0.313	0.308	0.298	0.011	0.60	0.91
10-14 wk	0.330	0.358	0.295	0.011	0.12	0.03
0-6 wk (Growing)	0.404	0.421	0.409	0.005	0.77	0.23
6-14 wk (Finishing)	0.322	0.337	0.297	0.008	0.28	0.18
Overall	0.351	0.372	0.342	0.006	0.50	0.07

SEM, standard error of the means.
[1)] 0.96, 4 growing-finishing pigs/pen (0.96 m^2/pig); 0.80, 5 growing-finishing pigs/pen (0.80 m^2/pig); 0.69, 6 growing-finishing pigs/pen (0.69 m^2/pig).

Table 3. Effect of different space allowance on serum cortisol and BUN concentration

Criteria	Treatment[1)]			SEM	p-value	
	0.96	0.80	0.69		Linear	Quadratic
Cortisol (μg/dL)						
Initial	2.7	2.7	2.7	-	-	-
3 wk	1.4	2.4	2.6	0.432	0.453	0.773
6 wk	2.0	2.0	3.4	0.689	0.209	0.159
10 wk	3.6	3.7	4.2	0.396	0.557	0.703
14 wk	2.3	3.3	4.0	0.498	0.046	0.108
Blood urea nitrogen (mg/dL)						
Initial	11.8	11.8	11.8	-	-	-
3 wk	12.3	13.7	10.8	0.467	0.316	0.424
6 wk	12.5	15.3	15.8	0.970	0.328	0.145
10 wk	13.8	15.5	14.0	0.342	0.673	0.011
14 wk	13.7	11.0	11.6	0.545	0.781	0.681

BUN, blood urea nitrogen; SEM, standard error of the means.
1) 0.96, 4 growing-finishing pigs/pen (0.96 m^2/pig); 0.80, 5 growing-finishing pigs/pen (0.80 m^2/pig); 0.69, 6 growing-finishing pigs/pen (0.69 m^2/pig).

observed on G/F ratio in late finishing period (p<0.03) and overall period (p = 0.07).

Hematological analysis

Space allocation did not affect BUN concentration in growing-finishing pigs, whereas, serum cortisol level linearly elevated with space allowance decreased (p<0.05) in 14 week (Table 3).

The effect of different space allowance on immunological parameters are shown in Table 4. There were no detectible differences in serum IgA concentration in 3, 6, 10, and 14 weeks, whereas IgG was linearly increased as space allocation increased in 10 and 14 weeks (p<0.01 and p<0.02, respectively).

Pork quality

The more pigs were crowded, the less pH of pork in 24 hour postmortem of carcass (p<0.01, Table 5). In addition, there was linear increase of shear force with decreased space allowance (p<0.01, Table 6).

Mortality and days to market weight

The effects of different space allowance on mortality and days to market weight are presented in Table 7. Whilst the pigs reared in 0.96 m^2 showed the shortest days to market weight (176 days), pigs in 0.69 m^2 recorded the longest days to market weight (208 days). Similarly, one pig (1 in growing phase) died in 0.96 treatment, but seven pigs (2 in growing, and 5 in late finishing phase, respectively) died in 0.69 treatment during the overall experimental period.

DISCUSSION

The data from the current experiment showed that there were no effect of space allocation on ADFI, ADG, and BW in growing

Table 4. Effect of different space allowance on immunological response in growing-finishing pigs

Criteria	Treatment[1)]			SEM	p-value	
	0.96	0.80	0.69		Linear	Quadratic
IgG (mg/dL)						
Initial	2.12	2.12	2.12	-	-	-
3 wk	2.61	4.34	2.60	0.605	0.98	0.16
6 wk	2.52	5.25	4.24	0.570	0.16	0.12
10 wk	5.27	4.22	2.27	0.414	0.01	0.52
14 wk	4.04	4.03	2.56	0.266	0.02	0.12
IgA (mg/dL)						
Initial	2.12	2.12	2.12	-	-	-
3 wk	2.52	2.31	2.21	0.253	0.65	0.93
6 wk	2.84	4.87	3.41	0.360	0.54	0.45
10 wk	5.06	3.25	3.87	0.423	0.25	0.11
14 wk	4.82	5.52	5.09	0.385	0.50	0.55

SEM, standard error of the means; IgG, immunoglobulin G; IgA, immunoglobulin A.
1) 0.96, 4 growing-finishing pigs/pen (0.96 m^2/pig); 0.80, 5 growing-finishing pigs/pen (0.80 m^2/pig); 0.69, 6 growing-finishing pigs/pen (0.69 m^2/pig).

Table 5. Effect of different space allowance on pork pH and lightness

Criteria	Treatment[1)]			SEM	p-value	
	0.96	0.80	0.69		Linear	Quadratic
pH						
1 h	5.87	5.87	5.88	0.02	0.85	0.92
24 h	5.68	5.67	5.62	0.01	0.01	0.09
CIE value L*						
1 h	40.19	39.76	40.79	40.25	0.85	0.79
24 h	48.02	45.83	46.50	46.78	0.67	0.29
CIE value a*						
1 h	1.54	1.56	1.70	1.60	0.23	0.81
24 h	4.00	3.51	4.10	3.87	0.28	0.86
CIE value b*						
1 h	3.93	3.82	3.94	3.90	0.23	0.98
24 h	6.54	5.86	6.27	6.23	0.23	0.48

SEM, standard error of the means; CIE, Commission Internationale de l'Eclairage.
1) 0.96, 4 growing-finishing pigs/pen (0.96 m^2/pig); 0.80, 5 growing-finishing pigs/pen (0.80 m^2/pig); 0.69, 6 growing-finishing pigs/pen (0.69 m^2/pig).

Table 6. Effect of different space allowance on proximate analysis and physiochemical properties of pork

Criteria	Treatment[1)]			SEM	p-value	
	0.96	0.80	0.69		Linear	Quadratic
Proximate analysis of loin meat						
Dry matter (%)	72.12	71.57	71.33	0.18	0.49	0.32
Crude protein (%)	23.23	23.15	24.11	0.24	0.07	0.50
Crude fat (%)	2.37	3.24	3.06	0.16	0.41	0.65
Crude ash (%)	1.34	1.46	1.39	0.11	0.39	0.19
Physiochemical property						
Cooking loss (%)	33.89	32.09	33.30	0.10	0.46	0.48
WBS (kg/0.75 cm^3)	4.72	5.16	5.38	0.33	0.01	0.49
WHC (%)	57.03	56.48	55.26	0.41	0.87	0.69

SEM, standard error of the means; WBS, Wamer-Bratzler shear force; WHC, water holding capacity.
1) 0.96, 4 growing-finishing pigs/pen (0.96 m^2/pig); 0.80, 5 growing-finishing pigs/pen (0.80 m^2/pig); 0.69, 6 growing-finishing pigs/pen (0.69 m^2/pig).

phase. Previous studies have insisted that there are various reasons for the effect of space allowance on growth performance in growing-finishing pigs; reduction in feed intake [11] and changes of behavioral requirement [12]. Brumm et al [3] stated that growing-finishing pigs reared in less than optimal space diminished feed intake, resulting in a reduction in ADG. More recently, White et al [13] reported that reducing stocking density from 0.93 to 0.66 m^2/pig resulted in 4.0% less BW, 17.0% less ADG, and 10.7% less ADFI. This finding is in agreement with our study that reduced space allowance from 0.96 to 0.69 m^2/pig resulted in less ADG and BW (17.0% and 12.1%, respectively) which might be associated with 14.7% less ADFI.

The coefficient value (k) of space allowance (A, m^2/pig) can be expressed using the equation reported by Petherick et al [14]: $A = k \times BW^{0.667}$. Several countries legislated or recommended minimum space requirement for pigs using this formula, which varied from 0.028 to 0.034 for growing-finishing pigs [15]. Moreover, recent study of Gonyou et al [6] demonstrated the relationship between space allowance and ADFI using broken-line analysis. Additionally, the range of critical coefficient value (k) determined by nonlinear analysis for growing-finishing pigs on partial slats was from 0.0357 to 0.0358 ($p<0.03$ and $p<0.01$, respectively). Our analysis resulted in an approximation of the critical value of $k = 0.045$ for 0.69 treatment (0.69 m^2/pigs) in growing phase, whereas critical value of $k = 0.033$ was obtained the in finishing phase. We assumed that growing pigs occupying 0.69 m^2 per pig was enough to grow without detrimental effects. This is also supported by the finding that the higher lipid accretion of pigs reared in the spacious pen is due to their higher feed intake, which, when in excess of the energy requirement for protein deposition and maximal lean gain, results in increased accretion ratio of lipid:protein [16].

Cortisol is a steroid hormone or glucocorticoid produced by the adrenal gland and released in response to stress. Generally, a poor welfare situation could lead to extreme stress to animals. Blood cortisol concentration has been the most common physiological parameter used to measure farm animal welfare [17], although the measurement suffers from diurnal variations and sample collection artifacts [18]. The current study showed that space allowance significantly influenced concentration of serum cortisol. This is consistent with the study of Zhang et al [19] who confirmed that the linearly increased cortisol concentration was in relation to higher stress in the pigs with 0.38 m^2 per pig than in those with 0.64 m^2. These results suggest that a chronic stress response as implied by the linear increase of cortisol concentrations with the higher stocking density may have a detrimental effect on growth performance.

The immune system serves the defense against the stress response in order to maintain homeostasis [20]. Serum IgG and IgA, widely used as an index of humoral immune parameters, are the major immunoglobulins in the extravascular compartment acting against pathogenic viruses and microorganisms [21].

Table 7. Effect of different space allowance on mortality and days to market weight

Criteria	Treatment[1)]			SEM	p-value	
	0.96	0.80	0.69		Linear	Quadratic
Days to market weight (110 kg)	176.04	183.93	207.78	19.724	0.01	0.79
Mortality (%, head)						
Growing phase	0.0 (0)	6.7 (2)	5.5 (2)	-	-	-
Finishing phase	4.1 (1)	7.1 (2)	14.7 (5)	-	-	-
Overall	4.1 (1)	13.3 (4)	19.4 (7)	-	-	-

SEM, standard error of the means.
1) 0.96, 4 growing-finishing pigs/pen (0.96 m^2/pig); 0.80, 5 growing-finishing pigs/pen (0.80 m^2/pig); 0.69, 6 growing-finishing pigs/pen (0.69 m^2/pig).

Recent studies by Woof et al [22] have concluded that where there is limited antigen, IgA is able to trigger effector functions that have the potential to destroy micro-organisms and mammalian cells by inhibiting complement activation. Considering our experimental condition, it seems likely that there were enough antigens to properly activate IgA in both treatments. The study of Tuchscherer et al [23] indicates that complement activation of IgG effectively provides the organism with a first line of non-specific humoral defense against infections before the immune response. Several studies noted that psychological stress can modulate the activation of the complement system [24]. Consequently, chronic stress derived from decreased space allowance might lead to nutritional disruption, resulting in the suppression of the IgG function in the present study.

Previous studies on the influence of space allowance on pork quality characteristics are prone to conflict, because most of studies compared different production systems rather than different environments with in the same system [25]. Brumm et al [3] found that different space allowance did not influenced carcass yield in grow to finish production systems. In contrast, earlier studies of Warriss et al [26] reported that pigs housed in higher density were more likely to produce paler meat than those at lesser density. Enfält et al [27] found a lower ultimate pH, higher drip loss, increased shear force values, and reduced intramuscular fat for outdoor compared to indoor reared pigs. Recent findings of Liorancas et al [25] reported that offering spacious conditions compared to commercial conditions resulted in higher muscle pH 24 hours postmortem, which is concordant with our result. The pH change is a very critical factor to determine pork quality. It has been acknowledged that initial pH is regarded as an indicator of PSE (pale, soft, and exudative) and the final pH is regarded as an estimation of DFD (dark, firm, and dry) pork [28]. The data from our result suggest that decreased spacial allocation increased carcass pH changes. One possible explanation is due to a higher blood cortisol level. Higher levels of blood cortisol were associated with higher pork temperatures, resulting in significantly lower ultimate pH values [29]. Thus, it is more susceptible to develop rapid rigor mortis [30]. Higher cooking loss and more water holding capacity [31] were observed in carcasses with rapid development of rigor mortis. In agreement with those findings, in this study, decreasing muscle glycolytic potential originated from chronic stress negatively influenced the rate of pH decline, resulting a detrimental effect on pork quality.

In the present study, higher mortality was observed in the pigs occupying 0.69 m^2, whereas 0.96 m^2 was the lowest. In agreement, numerous studies have shown that morbidity levels increase with a decrease in floor space [32]. More recently, Hamilton et al [4] stated that there was a trend for the mortality to be higher for pigs reared in the restricted than the unrestricted floor space.

Days to market weight per pig was shorter in the pigs reared in the largest (0.96 m^2) space allowance, but longest in the pigs reared in the small (0.69 m^2) space allowance. This finding is rather different from previous studies where the production and economic measures per pig improve with increased space allowance, the production per unit area or at a system level often still declines [6], which implies that the optimum from the pig and producers' perspective are different [33]. If policy makers make changes that benefit pig welfare, for example, by increasing space allowance, this can result in reduced margins for producers, unless they can obtain a price premium [34]. We cannot characterize increased space allowance as increasing total marginal profits, because we only measured days to market weight. Thus, multi-dimensional analysis is required to understand of the better production system in order to reduce economic loss of the farm.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

ACKNOWLEDGMENTS

This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Agri-Bio industry Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA; 314022-3).

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