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Short Communication

Effects of stocking density on growth performance, blood parameters and immunity of growing pigs

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ABSTRACT

This study was conducted to evaluate the effects of different stocking densities on growth performance, blood parameters, intestinal morphology and intestinal immunity of growing pigs. A total of 288 male pigs (44.35 ± 0.50 kg) were randomly assigned to groups with stocking densities of 2.46, 1.23 and 0.82 m^2/pig for a month. The results showed that there was no significant difference on growth performance among groups. Pigs in the stocking density of 0.82 m^2/pig had the lowest backfat thickness and spleen weight index among groups ($P < 0.05$). With increasing stocking density, the serum concentrations of blood urea nitrogen (BUN), transglutaminase (TGG), alkaline phosphatase (AKP) and immunoglobulin A (IgA) were increased, and albumin (ALB), albumin-to-globulin ratio (ALB:GLO), insulin-like growth factor-1 (IGF-1) and immunoglobulin (IgM) were decreased ($P < 0.05$), and cortisol tended to increase and glucose tended to decrease ($0.05 < P < 0.1$). Compared with the stocking density of 2.46 m^2/pig, the ileal villus height and jejunal villus width decreased in stocking densities of 1.23 and 0.82 m^2/pig ($P < 0.05$). The duodenal villus height and ileal villus width in the stocking density of 0.82 m^2/pig were the lowest among 3 groups ($P < 0.05$). The content of immunoglobulin A in duodenum, jejunum and ileum mucosa increased along with increasing density ($P < 0.05$). The contents of interleukin (IL)-2 in the spleen or liver and IL-10 in the spleen were higher in the stocking density of 0.82 m^2/pig than in other 2 groups. These results showed that stocking density could affect the metabolism, intestinal morphology, and immunity of growing pigs and 1.23 m^2/pig may be the suitable stocking density for the growing pigs in the present study.

1. Introduction

With commercialization and intensive development of swine industry, pigs are often stocked at a high density for efficient management, improving pen space utilization and profitability (Guise et al., 1998). However, as one of the important parameters of pork production, stocking density affects the environment as well as the physical and behavior of pigs (Larsen et al., 2017). High stocking

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density causes scarcity of living space and feed and increases environmental burden on pigs, such as the existence of noxious gases and occurrence of respiratory and digestive tract diseases (Cornale et al., 2015). Thus, pigs under a high stocking density falls into severe environmental, social and psychological stress.

Severe stress adversely affects animal health. Firstly, stress can affect the growth performance owing to the increased energy consumption (Marco-Ramell et al., 2011). Furthermore, high stocking densities might increase the competition for feed, living space and other living resources (Zhang et al., 2013). Secondly, immune and health status are seriously affected by chronic stress caused by high stocking densities (Griffin, 1989). With an increase in stocking density, the temperature and humidity also increase, thereby affecting the ventilation conditions. Harmful gases in a piggery mainly include ammonia (NH_3), carbon dioxide (CO_2), carbon monoxide (CO) and hydrogen sulfide (H_2S). These gases can directly and adversely affect the respiratory system of pigs and cause toxic reactions or an immunity reduction after exposure (Beline et al., 2008).

A previous study calculated that gain is maximized when the floor area allowance is approximately 0.048 m^2/kg $BW^{0.667}$ kg (Kornegay and Notter, 1984). Gonyou and Stricklin (1998) showed that housing densities less than 0.76 m^2/pig reduce average daily gain and average daily feed intake in grow-finish swine. The effect of density or group size on feed intake has been shown to vary, especially for older pigs (Gonyou and Lou, 2000). Previous studies on the effects of stocking density on pigs mainly focused on growth performance, behavior habit, and animal welfare (Anderson et al., 2012). The health of growing pigs is vital to overall growth stages and the economic efficiency of swine industry (Kim et al., 2018). The understanding of how stocking density affects the health of pigs requires a detailed evaluation on growing pigs. Therefore, this study aimed to investigate the effects of stocking density on growth performance, blood parameters and intestinal morphology of growing pigs.

2. Materials and methods

This study was conducted according to the guidelines for the treatment of animals as approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences.

2.1. Animals, housing and diets

For the experiment, 288 male pigs (Duroc × [Landrace × Large White], average BW = 44.35 ± 0.50 kg, age = 95 ± 5 d) were housed for a month (30 d) under 3 stocking densities, but in the same area of the piggery. The housing area was 19.76 m^2 (5.2 m wide by 3.8 m long). Every treatment had 6 replications. These pigs were randomly assigned to 3 treatments: 1) a high density (HD; 0.82 m^2/pig) group, in which 24 pigs were housed in each pen; 2) a normal density (ND; 1.23 m^2/pig) group, in which 16 pigs were housed in each pen; and 3) a low density (LD; 2.46 m^2/pig) group, in which 8 pigs were housed in each pen. A single nipple waterer and feeder were placed in every pen. All pigs were provided free access to water and feed. The basal diet was formulated to meet the nutrient requirement for growing pigs (NRC, 2012). The growth performance was measured based on average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F:G) (Xiong et al., 2015). The BW of pigs in each pen was recorded at the beginning of the experiment and before feeding on d 30.

2.2. Sample collection and analyses

At the completion of the test on 30 d, 2 pigs close to the average BW of the pen were selected from every pen for blood sampling after they were fasted for 12 h. Blood samples were collected by cava venipuncture in 10-mL tubes without anticoagulant (for serum). Serum samples were obtained by centrifugation at 2,000 × *g* for 10 min at room temperature and frozen at −80 °C until assay.

The following blood parameters were monitored: cortisol, blood urea nitrogen (BUN), total cholesterol (TCH), triglyceride, glucose, transglutaminase (GGT), alanine aminotransferase (ALT), alkaline phosphatase (AKP), high density lipoprotein (HDL), low density lipoprotein (LDL), total protein, albumin (ALB), globulin (GLO), albumin-to-globulin (ALB:GLO) ratio, insulin-like growth factor-1 (IGF-1), growth hormone (GH), immunoglobulin M (IgM), immunoglobulin A (IgA) and IgG. Above parameters were analyzed using TBA-120FR (Toshiba). Interleukin (IL)-1β, IL-2, IL-10, and secretory immunoglobulin A (SIgA) were analyzed using ELISA kits (Cusabio Biotech Co., Ltd., Hubei, China).

2.3. Measurement of backfat thickness

After blood sample were obtained, the pigs were euthanized and exsanguinated. A vernier caliper was used to measure the backfat thickness at the same site in each pig at 4 cm from the dorsal midline between the 10th to 11th ribs.

2.4. Organ and tissue collection and analyses

Parts of the liver and spleen tissues were immediately collected and snap-frozen in liquid nitrogen and stored at − 80 °C for later analysis. Scraping the intestinal mucosa of duodenum, jejunum and ileum separately with a slide after rinsing the intestinal contents with physiological saline, and then they were stored at −80 °C as the above steps. Duodenum was collected from the junction of the stomach and small intestine and ileum was collected anterior to the ileocecal junction, whereas jejunum was dissected in the middle of the small intestine.

The liver, spleen and the intestinal mucosa of duodenum, jejunum and ileum (approximately 100 mg) were homogenized in a 9-fold volume of PBS (0.1 mol/L, pH 7.2) and then centrifuged at 3,000 × *g* for 10 min at 4 °C to obtain the supernatant. The contents of IL-1β, IL-2, IL-10 and SIgA were measured using ELISA kits (Cusabio, Wuhan, China).

2.5. Statistical analysis

The results are expressed as means ± SEM. Data were statistically analyzed using one-way analysis of variance to determine differences between groups. Normality of the data was assessed using a Shapiro–Wilk test and homogeneity of variance was also verified using the Levene's test. All statistical analyses were conducted using the Statistical Package for Social Science (SPSS for Windows; v19.0, USA). Differences were considered statistically significant when $P < 0.05$ and highly significant at the level of $P < 0.01$, and tendencies were accepted when $0.05 < P < 0.1$.

3. Results

3.1. Growth performance and backfat thickness

No significant differences were noted among ADG, ADFI, or F:G ratio of growing pigs after 30 d of treatment ($P > 0.05$) and pigs in the HD group had the lowest backfat thickness ($P < 0.01$; Table 1).

Table 1
Effects of stocking density on the growth performance of growing pigs (kg).[1]

Item	Stocking density[2]			*P*-value
	LD	ND	HD	
Starting weigh	44.35 ± 0.49	44.43 ± 0.49	44.27 ± 0.51	0.998
Ending weigh	70.45 ± 0.36	69.63 ± 1.35	68.87 ± 0.64	0.636
ADFI	2.17 ± 0.05	2.15 ± 0.05	2.12 ± 0.05	0.822
ADG	0.87 ± 0.01	0.84 ± 0.03	0.82 ± 0.01	0.311
F:G, g/g	2.51 ± 0.07	2.56 ± 0.05	2.58 ± 0.08	0.753
Backfat thickness, mm	14.56 ± 0.33[a]	13.41 ± 0.98[a]	9.17 ± 0.65[b]	0.001

LD = low stocking density; ND = normal stocking density; HD = high stocking density; ADFI = average daily feed intake; ADG = average daily gain; F:G = the ratio of average daily feed intake to average daily gain.
[a,b] Within a row, means with different superscript letters are significantly different ($P < 0.05$).
[1] Data represent means ± SEM ($n = 6$).
[2] LD, 2.46 m^2/pig; ND, 1.23 m^2/pig; HD, 0.82 m^2/pig.

Table 2
Effects of stocking density on organ weight index of pigs (g/kg).[1]

Item	Stocking density[2]			*P*-value
	LD	ND	HD	
Liver	19.73 ± 0.51	21.28 ± 1.22	19.04 ± 0.36	0.157
Kidney	1.96 ± 0.10	2.10 ± 0.16	1.87 ± 0.09	0.425
Spleen	1.84 ± 0.09[a]	1.49 ± 0.15[ab]	1.34 ± 0.13[b]	0.033
Heart	4.09 ± 0.09	3.71 ± 0.39	4.22 ± 0.14	0.350

LD = low stocking density; ND = normal stocking density; HD = high stocking density.
[a,b] Within a row, means with different superscript letters are significantly different ($P < 0.05$).
[1] Data represent means ± SEM ($n = 6$).
[2] LD, 2.46 m^2/pig; ND, 1.23 m^2/pig; HD, 0.82 m^2/pig.

3.2. Organ indexes

The organ index of spleen was decreased significantly with the increase of stocking density ($P < 0.05$), but there was no significant difference in organ indexes of others ($P > 0.05$; Table 2).

3.3. Serum biochemical parameters

The present study showed that compared with LD group, pigs in ND or HD group showed increased serum BUN, triglyceride, GGT, AKP, IgA ($P < 0.05$), cortisol ($0.05 < P < 0.1$), and showed decreased serum IgM ($P < 0.05$). However, serum glucose content was decreased compared with that in the LD group ($0.05 < P < 0.10$). The pigs in the HD group had the lowest content of ALB, ALB-to-GLO ratio and IGF-1 ($P < 0.01$). No significant differences were noted for the content of TCH, AKP, HDL and LDL among the groups ($P > 0.05$; Table 3).

3.4. Intestinal morphology

Compared with LD group, the ileal villus height and jejunal villus width in ND or HD group decreased ($P < 0.05$). The duodenal villus height and ileal villus width in stocking densities of 0.82 m^2/pig were the lowest in 3 groups ($P < 0.05$). There were no significant differences in crypt depth or villus height-to-crypt depth ratio among 3 groups (Table 4).

3.5. Cytokines

The contents of IL-2 in the spleen or liver and IL-10 in the spleen in HD group were higher than those in LD or ND group ($P < 0.05$; Table 5). The content of SIgA in duodenum, jejunum and ileum mucosa increased significantly ($P < 0.05$) with increasing density.

4. Discussion

The results showed that no significant differences in ADG, ADFI, F:G ratio, or serum GH content were noted among 3 groups in the present study. However, high stocking density decreased significantly the backfat thickness of pigs. Previous studies showed that stocking density or group size has been shown to affect the growth performance and health status of pigs (White et al., 2008; Funk et al., 2007). Pigs raised in 1.3 m^2/pig showed a better growth performance compared with those in 1.0 m^2/pig (Nannoni et al.,

Table 3
Effects of stocking density on the serum physiological and biochemical indexes in growing pigs.[1]

Item	Stocking density[2]			*P*-value
	LD	ND	HD	
BUN, mmol/L	2.49 ± 0.18[b]	3.82 ± 0.45[a]	3.94 ± 0.40[a]	0.023
TCH, mmol/L	2.28 ± 0.03	2.28 ± 0.03	2.30 ± 0.05	0.376
Triglyceride, mmol/L	0.28 ± 0.03[b]	0.31 ± 0.04[b]	0.49 ± 0.08[a]	0.026
Glucose, mmol/L	5.19 ± 0.16	5.11 ± 0.29	4.45 ± 0.22	0.063
GGT, U/L	31.17 ± 2.34[b]	43.33 ± 2.93[a]	46.00 ± 3.74[a]	0.008
AKP, U/L	154.67 ± 8.80[c]	178.83 ± 5.52[b]	201.83 ± 7.86[a]	0.002
ALT, U/L	45.50 ± 3.01	51.83 ± 4.39	54.67 ± 1.71	0.155
HDL, mmol/L	1.18 ± 0.09	1.20 ± 0.08	1.19 ± 0.04	0.958
LDL, mmol/L	1.54 ± 0.05	1.48 ± 0.09	1.55 ± 0.19	0.744
Total protein, g/L	66.80 ± 1.10	64.37 ± 1.65	62.95 ± 0.94	0.166
ALB, g/L	41.23 ± 0.85[a]	39.83 ± 0.62[b]	36.73 ± 0.64[b]	0.001
GLO, g/L	26.68 ± 1.24	25.73 ± 1.08	23.90 ± 0.92	0.228
ALB-to-GLO ratio	1.80 ± 0.08[a]	1.59 ± 0.05[b]	1.37 ± 0.06[c]	0.001
IgM, mg/dL	66.80 ± 5.16[a]	58.22 ± 4.35[b]	49.90 ± 3.54[b]	0.050
IgA, mg/dL	0.70 ± 0.10[b]	0.87 ± 0.02[ab]	1.02 ± 0.07[a]	0.023
IgG, mg/dL	298.22 ± 19.06	276.26 ± 10.17	266.43 ± 12.81	0.312
Cortisol, mmol/L	97.80 ± 10.21	121.00 ± 12.27	139.33 ± 8.35	0.053
IGF-1, μg/L	206.61 ± 15.48[a]	208.17 ± 20.45[a]	144.67 ± 11.18[b]	0.001
GH, ng/mL	0.06 ± 0.013	0.06 ± 0.006	0.07 ± 0.008	0.993

LD = low stocking density; ND = normal stocking density; HD = high stocking density; BUN = blood urea nitrogen; TCH = total cholesterol; GGT = transglutaminase; AKP = alkaline phosphatase; ALT = alanine aminotransferase; HDL = high density lipoprotein; LDL = low density lipoprotein; ALB = albumin; GLO = globulin; IgM = immunoglobulin M; IgA = immunoglobulin A; IgG = immunoglobulin G; IGF-1 = insulin-like growth factor-1; GH = growth factor.
[a, b, c] Within a row, means with different superscript letters are significantly different ($P < 0.05$).
[1] Data represent means ± SEM ($n = 6$).
[2] LD, 2.46 m^2/pig; ND, 1.23 m^2/pig; HD, 0.82 m^2/pig.

Table 4
Effects of stocking density on intestinal morphology in growing pigs (μm).[1]

Item	Stocking density[2]			*P*-value
	LD	ND	HD	
Villus height				
Duodenum	533.96 ± 21.15[ab]	568.99 ± 12.44[a]	519.53 ± 14.77[b]	0.047
Jejunum	414.08 ± 12.97	399.78 ± 10.30	409.57 ± 9.25	0.645
Ileum	489.48 ± 10.39[a]	431.90 ± 6.81[b]	417.38 ± 9.79[b]	0.000
Crypt depth				
Duodenum	387.54 ± 16.66	370.90 ± 8.89	393.01 ± 17.30	0.441
Jejunum	274.54 ± 8.68	258.41 ± 7.76	263.43 ± 7.13	0.359
Ileum	202.23 ± 5.29	187.29 ± 5.06	188.00 ± 7.88	0.118
Villus width				
Duodenum	178.07 ± 12.41	181.66 ± 5.58	193.26 ± 7.54	0.407
Jejunum	175.33 ± 5.47[a]	153.65 ± 5.86[b]	151.66 ± 6.27[b]	0.012
Ileum	147.03 ± 4.22[a]	149.26 ± 4.00[a]	131.55 ± 4.15[b]	0.010
Villus height-to-crypt depth, μm/μm				
Duodenum	1.46 ± 0.08	1.59 ± 0.06	1.42 ± 0.07	0.113
Jejunum	1.59 ± 0.08	1.62 ± 0.06	1.64 ± 0.06	0.866
Ileum	2.55 ± 0.08	2.43 ± 0.07	2.39 ± 0.09	0.330

LD = low stocking density; ND = normal stocking density; HD = high stocking density.
[a,b] Within a row, means with different superscript letters are significantly different ($P < 0.05$).
[1] Data represent means ± SEM ($n = 6$).
[2] LD, 2.46 m^2/pig; ND, 1.23 m^2/pig; HD, 0.82 m^2/pig.

2019). Group size significantly decreased feed intake and growth rate for growing pigs with the same stocking density (Gomez et al., 2000). Reducing stocking density from 0.93 to 0.66 m^2/pig resulted in the reduction of BW by 4.0%, ADG by 17.0%, ADFI by 10.7%, and G:F ratio by 7.8% (White et al., 2008). This discrepancy on growth performance might be attributed to the differences in feeding period (the size of the pig) or feeding time. Chemical composition of the carcasses of growing pigs is highly associated with backfat thickness and carcass weight (Aziz and Ball, 1995). A reduction in backfat thickness was accompanied by a decrease in the fat content of the subcutaneous fat depot (Fortin and Elliot, 1985). The present study showed that the fat content of the subcutaneous fat depot in growing pigs decreased with increasing stocking density.

Serum biochemical parameters are useful indices for monitoring the health and physiological condition of animals. In the present study, although the stocking density did not affect the growth performance, it affected the serum parameters. Serum BUN concentration has been used as an indicator of amino acid utilization efficiency and is related to the status of protein metabolism and retained dietary nitrogen in animals (Jia et al., 2016). In our study, the content of BUN of pigs in the ND or HD group was significantly higher than that of the LD group. These results indicate that ND or HD might result in unbalanced amino acids and thus decreased protein synthesis (Lv et al., 2018). This could be because of stress, e.g. higher densities can induce the physiological changes to help an individual cope with the stressor (Padgett and Glaser, 2003). The content of serum triglyceride can be used to determine the body fat metabolism (Meng et al., 2017). The present study showed that the growing pigs in the HD group had increased serum triglyceride. Blood glucose is one of the important energy sources for all life activities (Barb et al., 1991). The present study showed that the glucose content also tended to decrease with increasing stocking density, which may indicate a lower rate of glucose used with the increasing stocking density (Lv et al., 2018). With an increase in stocking density, the content of GGT, AKP, ALB and ALB-to-GLO ratio significantly changed. Serum GGT, AKP and ALB are mainly secreted by the liver and reflect body health (Li et al., 2017). Albumin plays a role in the maintenance of plasma osmotic pressure and metabolism of nutrients (Lee et al., 2009). These results indicated that stocking density affects the liver function and physiological condition of growing pigs.

Cortisol is traditionally used for assessing stress in animals (Warriss et al., 1998). It regulates growth, immunity and intermediary metabolism (Chrousos, 2007). Compared with LD group, the serum cortisol content tended to increase in ND or HD group,

Table 5
Effects of stocking density on cytokines of the intestinal mucosa, spleen and liver in growing pigs (pg/mg).[1]

Item	Stocking density[2]			*P*-value
	LD	ND	HD	
IL-1β				
Duodenum	4.98 ± 0.81	8.38 ± 2.35	9.58 ± 2.87	0.280
Jejunum	6.29 ± 1.27	8.29 ± 0.96	10.55 ± 4.41	0.462
Ileum	5.79 ± 1.15	7.79 ± 1.63	8.11 ± 1.29	0.500
Liver	276.84 ± 36.41	362.19 ± 66.94	419.89 ± 77.99	0.287
Spleen	5.78 ± 0.65	5.79 ± 0.64	5.27 ± 0.37	0.135
IL-2				
Duodenum	48.15 ± 11.68	61.40 ± 8.88	72.40 ± 11.10	0.301
Jejunum	62.19 ± 16.95	67.85 ± 8.58	76.94 ± 7.74	0.716
Ileum	64.84 ± 19.43	69.95 ± 4.25	73.55 ± 12.18	0.894
Liver	1,331.74 ± 153.11[b]	1,606.14 ± 95.43[ab]	1,809.28 ± 83.64[a]	0.034
Spleen	54.53 ± 4.41[b]	66.82 ± 6.09[ab]	76.44 ± 4.46[a]	0.038
IL-10				
Duodenum	10.39 ± 1.83	16.86 ± 4.16	13.47 ± 1.98	0.285
Jejunum	11.39 ± 2.34	14.93 ± 1.79	17.84 ± 6.97	0.516
Ileum	11.29 ± 2.25	14.48 ± 2.96	21.23 ± 5.74	0.237
Liver	395.73 ± 31.85	523.98 ± 70.02	552.61 ± 52.35	0.127
Spleen	11.52 ± 0.90[b]	9.69 ± 1.38[b]	19.88 ± 0.92[a]	0.010
SIgA, μg/mg				
Duodenum	8.84 ± 0.98[b]	13.12 ± 2.18[ab]	17.02 ± 2.38[a]	0.033
Jejunum	14.88 ± 0.68[b]	17.66 ± 2.72[ab]	24.05 ± 2.38[a]	0.050
Ileum	13.18 ± 3.87[b]	23.30 ± 2.69[ab]	33.53 ± 7.71[a]	0.048

LD = low stocking density; ND = normal stocking density; HD = high stocking density.
[a, b] Within a row, means with different superscript letters are significantly different ($P < 0.05$).
[1] Data represent means ± SEM ($n = 6$).
[2] LD, 2.46 m^2/pig; ND, 1.23 m^2/pig; HD, 0.82 m^2/pig.

indicating that pigs may have higher stress in the 2 groups than in LD group. The intense stress can lead to an immune dysfunction (Bornett et al., 2000). The spleen plays a vital role in the modulation of immune system (Tarantino et al., 2013). The results for relative weights of organs showed that the higher stocking density exerted adverse effects on spleen organ index. Serum IgA seems to be mostly dependent on cell activity in the bone marrow, spleen and lymph nodules (Drochner et al., 2004). With an increase in stocking density, serum IgA was also significantly increased, which indicates that a higher stocking density stimulates the production of serum IgA in growing pigs. However, serum IgM content was significantly reduced.

Secretory immunoglobulin A is mainly involved in mucosal immunity and plays an important role in protecting the whole body and keeping the environment stable (Sandin et al., 2016). SIgA content in duodenum, jejunum and ileum mucosa also significantly increased in our experiment. We speculated that pigs of the highest stocking density are exposed to more pathogenic microorganisms than those in the other 2 groups (Crivelli, 2001), thus stimulating the immune protection mechanism and promoting the secretion of SIgA on intestinal mucosa. Cytokines play an important role in the immune and inflammatory responses, and their balance is important for protection against infection (Warriss et al., 1998). The present study also showed that the secretion of IL-10 in the spleen or IL-2 in the spleen and liver in HD group increased. IL-10 is a recognized suppressor of inflammation and immunity, which has a wide range of anti-inflammatory effects and immunosuppressive activities (Walter, 2014). These findings suggest that the increased stocking density may induce an immune response in pigs by modulating the production of cytokines and antibodies.

5. Conclusion

In conclusion, the present study showed that stocking density could affect backfat thickness, serum metabolic parameters and the immunity index of growing pigs. The stocking density of 1.23 m^2/pig may be suitable for the growing pigs in the present study.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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