Impacts of Host Immunization on the Translocation of Intestinal Bacteria and Growth Performance in Weanling Piglets

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ABSTRACT : Effects of host immunization on bacterial translocation and growth performance in weanling piglets were studied. Twenty four barrows were assigned to one of two immunization treatments: Control group (CON: immunized with placebo) or Immunization group [IMMU: immunized with Antigen cocktail; Keyhole limpet hemocyanin (KLH), Ovalbumin (OA), and Tetanus toxoid (TT)]. On d0, piglets were weaned and intramuscularly immunized with 2 ml of placebo or Antigen cocktail, respectively. Antigen-specific Ig titers were determined by ELISA (Enzyme Linked ImmunoSorbent Assay). Ig titers to *E. coli*-derived lipopolysaccharides (LPS) were measured as the indicator of bacterial translocation. Ig titers to LPS were higher ($p<0.10$, 0.05 or 0.01) in CON group before immunization (d0), but the difference disappeared with time and IgA titers to LPS became higher ($p<0.05$) in IMMU group on d39. In IMMU group, IgG titers to LPS from d28 onwards showed positive correlations ($p<0.10$, 0.05, 0.01 or 0.001) with IgG titers to KLH from d11 onwards and with IgM titers to KLH from d7 onwards. Generally, growth performance was negatively related to IgG titers to LPS. Average daily gain for d28 to d35 showed negative correlations ($p<0.10$, 0.05, or 0.01) with IgG titers to LPS on d28 onwards in immunization group. These results reveal some evidences that host immunization might facilitate bacterial translocation and high humoral immune responses to LPS are negatively related with the growth performance. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 2 : 180-185*)

Key Words : Immunization, Bacterial Translocation, Lipopolysaccharide, Growth, Pig

INTRODUCTION

As reviewed by Wells (1990), normal intestinal bacteria can leave the intestinal lumen and enter extraintestinal sites by a process called bacterial translocation and cause various complicating infections. The gut is thought to have a specific defense system, termed gut barrier or colonization resistance (Van der Waaij et al., 1971, 1972a, 1972b) against unwanted bacteria which may involve immunity as well as bacterial antagonism. The mechanism involved in gut barrier or colonization resistance may be explained largely by two factors of microbial and host origin. Strictly anaerobic bacteria were thought to play a major role in the establishment of a high colonization resistance, thus preventing bacterial translocation of potentially pathogenic aerobic or facultative bacteria (Van der Waaij et al., 1971, 1972a, 1972b). The host factors include peristaltic movement, secretion of mucus, epithelial cell desquamation, the Gut Associated Lymphoid Tissue (GALT) producing sIgA, and nonspecific adherence blocking factors (Van der Waaij, 1989).

There are increasing direct experimental and indirect clinical evidences to indicate that intestinal barrier function may be lost, and translocation of bacteria and endotoxins may be facilitated in severely ill patients or animals suffering from burn trauma (Maejima et al., 1984; Deitch, 1990; Fukushima et al., 1994) and cancer (Tancrede and Andremont, 1985). Furthermore, it may be assumed that possible infections resulting from translocated bacteria could affect the nutritional and/or health status of animals, thus animal production, especially at the moment of high risk such as weaning period.

We have hypothesized that host immunization, like trauma, may enhance bacterial translocation by affecting intestinal barrier function. We have assumed that blood/systemic antibody titers against *E. coli*-derived lipopolysaccharide (LPS) may be the indicator of the intensity of bacterial translocation, since the blood LPS should be originated from the gut only unless *E. coli* or LPS itself are injected intravascularly or intramuscularly. Thus, this study was designed to investigate the impacts of immunization of the host on the translocation of intestinal bacteria and growth performance.

MATERIALS AND METHODS

Animals, housing and feeding

24 weanling barrows of approximately 4 weeks of age with a mean body weight of 7.7 ± 0.20 kg were divided into two groups based on litters and body weight, and assigned to one of two immunization treatments: control group (CON: immunized with placebo; 1:1 v/v Specoll : PBS) or immunization group [IMMU: immunized with Antigen cocktail; 1 mg Keyhole limpet hemocyanin (KLH), 4 mg ovalbumin (OA), and 15 Lf tetanus toxoid (TT) in a 1:1 v/v Specoll: PBS]. Piglets were intramuscularly immunized with 2 ml of placebo or Antigen cocktail on the day of weaning (d0) after transportation approximately 60 km to the respiration chambers. The piglets were obtained from

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the Research Institute for Pig Husbandry in Rosmalen, The Netherlands, and originated from Dutch Landrace, Finnish Landrace, and Great Yorkshire rotational-bred sows and Great Yorkshire terminal boars.

The experiment consisted of a 13-d balance period followed by a 28-d growing period. During the balance period, effects of immunization procedure on energy metabolism were studied and have been reported (Gentry et al., 1997). During the balance period of 13 days, each group was housed in a open circuit indirect climatic respiration chamber which has inner dimensions (available for animal) of 1.0 m×0.8 m×0.97 m in length×width×height (Verstegen et al., 1987). In the chambers the temperature was kept within thermoneutral zone for the pigs (28, 27, 26, and 25℃ on d0 to d3, d3 to d6, d6 to d10, and d10 to d13, respectively) with the relative humidity of about 65-70%. Air velocity was <0.20 m/s. The piglets were exposed to 12 h of light and 12 h of darkness in chambers as well as in growing period. The artificial light was on from 07:00 to 19:00. During the growing period, the piglets were housed in raised 1.2 m×1.5 m pens with iron slat flooring. The temperature was also kept within thermoneutral zone at approximately 24℃.

Piglets were allowed ad libitum access to feed, a commercial pelleted starter diet, and water. Determined gross energy (GE) and crude protein (CP) content were 16.6 kJ/g and 17.3%, respectively.

Measurements

For measuring antibody titers, blood samples were taken using coagulated blood tubes from the vena cava on d0, 3, 7, 11, 14, 18, 21, 25, 28, 32, 35, 39, and 42. Antigen-specific and anti-LPS antibody titers were determined by ELISA (Enzyme Linked ImmunoSorbent Assay).

A one-step or a two-step procedure was followed to determine systemic Ig total, IgG and IgM (one-step), and IgA (two-step), respectively. Briefly, serial dilutions of sera from all animals, including a (pooled) standard positive control serum, were added to antigen-coated wells of a microtiterplate (0.1 μg/ml KLH, 0.02 μg/ml TT, 1 μg/ml OA, 4 μg/ml LPS as 200 μℓ coating buffer per well). After incubation for 1 h at 37℃ and subsequent washing, a one step conjugation was performed. Conjugation consisted of incubation for 1 h with a 1:8000 diluted peroxidase (PO) conjugated rabbit anti-swine IgG (H+L) (R∝Sw-IgG (H+L)/PO, Kpl, Gaithersburg MD, USA) for total Ig, 1:8000 diluted rabbit anti-swine IgG Fc (R∝Sw-IgG (Fc)/PO, Nordic, Tilburg, The Netherlands) for IgG, or 1:8000 diluted goat anti-swine IgM Fc (G∝Sw-IgM (Fc)/PO, Nordic, Tilburg, The Netherlands) for IgM.

The two-step procedure consisted of incubation for 1 h with 1:6000 diluted mouse anti-swine IgA monoclonal antibody (M∝Sw-IgA, ID-DLO, Lelystad, The Netherlands), followed by 1-h incubation with 1:500 diluted rabbit anti-mouse antibody (R∝M/PO, Dakopatts, Glostrup, Denmark). After the wells were washed, tetramethylbenzidine (TMB, Sigma T2885) was added as a substrate. Colour development was stoped with 2.5 N sulphuric acid after 10 minutes. All absorbances were expressed relative to the absorbance curve of the standard positive control serum. The titer of antibodies to KLH, TT, OA, or LPS in a sample was the dilution that gave an extinction closest to the 50% of E_{max}, where E_{max} represents the highest mean extinction of a standard positive (pooled) serum.

Statistics

The effect of immunization on the traits was tested by means of F-tests using a split-plot model (GLM procedure; SAS, 1989), with values in time of an individual animal taken as repeated measurements:

$$Y_{ijk} = \mu + I_i + e_{1,\,ij} + T_k + (I * T)_{ik} + e_{2,\,ijk}$$

where, Y_{ijk} = a specific trait at immunization procedure i, animal j, and day period k; μ = overall mean; I_i = the effect of immunization procedure i (i = 1, 2); $e_{1,\,ij}$ = error term 1, which represents the random effect of animal j within immunization procedure i (j = 1, . . . ,12); T_k = the effect of day period k (k = 1, . . . , 13); and $e_{2,\,ijk}$ = error term 2, which represents the random effect within animal between day period.

The effect of immunization procedure on antibody responses was tested for significance against the random effect of animal nested within immunization procedure (error term 1). The effect of time (day period) and the interaction between time and immunization procedure were tested against the random effect between time period within animal (error term 2). Correlation coefficients (r) were obtained as Pearson coefficients.

RESULTS

Figure 1 shows Ig total titers to three immunogens (KLH, TT and OA) in IMMU group. Total Ig titers to KLH, TT and OA increased from around d7 and remained high throughout the observation period.

Systemic antibody responses to LPS are shown in Figures 2, 3 and 4. Ig titers to LPS decreased during the first three or four weeks. However, Ig total and IgG titers to LPS increased from 3rd week post immunization onwards in IMMU group, while those in CON group continuously decreased (figures 2 and 3). As shown in figure 4, anti-LPS IgA response increased on d28 in IMMU group, and was higher ($p<0.05$) than that in CON group on d39, in spite of the lower titer in IMMU group than that in CON group on d0 ($p<0.10$).

Tables 1 and 2 represent the correlation coefficients between Ig titers to KLH and Ig titers to LPS in IMMU group. In general, correlations between antibody responses to 3 immunogens (KLH, TT, OA) and those to LPS showed very similar tendency, so only the correlations with KLH were presented. As shown in tables 1 and 2, IgG titers to LPS from d28 onwards had significant positive correlations with IgG titers to

KLH from d11 onwards ($p<0.10$, 0.05 or 0.01), and with IgM titers to KLH from d7 onwards ($p<0.10$, 0.05, 0.01 or 0.001).

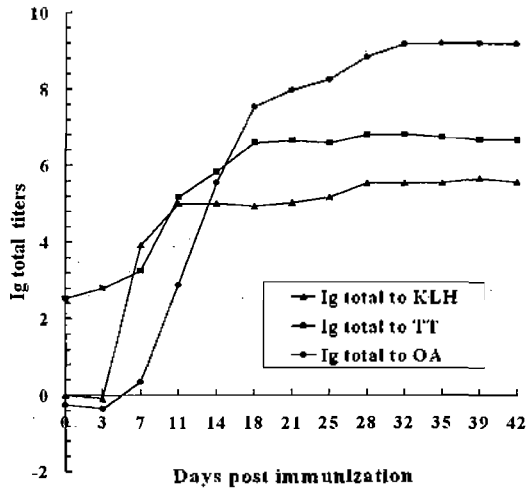

Figure 1. Changes of Ig total titers to 3 immunogens injected in immunization group. KLH: Keyhole Limpet Hemocyanin (SE = 0.294); TT: Tetanus Toxoid (SE = 0.270); OA: Ovalbumin (SE = 0.232)

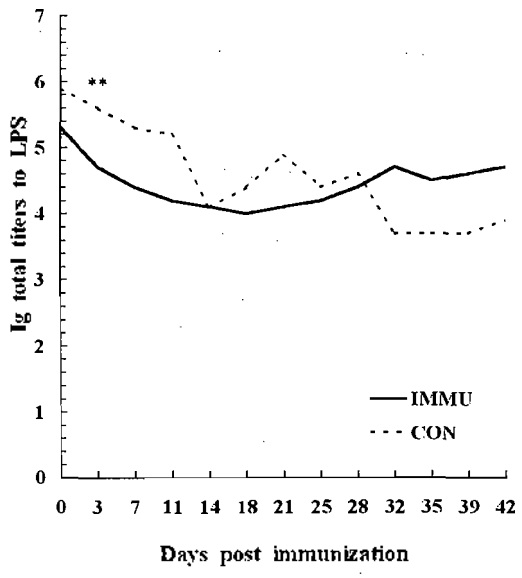

Figure 2. Ig total titers to lipopolysaccharide (LPS) in control (CON) and immunization (IMMU) group. SE_{IMMU} = 0.222, SE_{CON} = 0.260; ** $p<0.01$.

Effects of anti-LPS IgG responses on average daily gain (ADG) in IMMU group are shown in table 3. Generally, growth performance was negatively related with IgG titers to LPS. IgG titers to LPS on d11 had negative correlation ($p<0.05$) with ADG1 (d0 to d7; r = - 0.59), ADG2 (d7 to d14; r = - 0.61) and ADG6 (d35 to d42; r = - 0.60). Especially, a high negative correlation (r = - 0.77) was observed between ADG4 (d21 to d28) and IgG titers to LPS on d25 ($p<0.01$). ADG5 (d28 to d35) showed significant negative correlation with IgG titers to LPS from d28 onwards, especially the correlation coefficients were high on d39 (r = - 0.73, $p<0.01$) and on d42 (r = - 0.74, $p<0.01$), respectively.

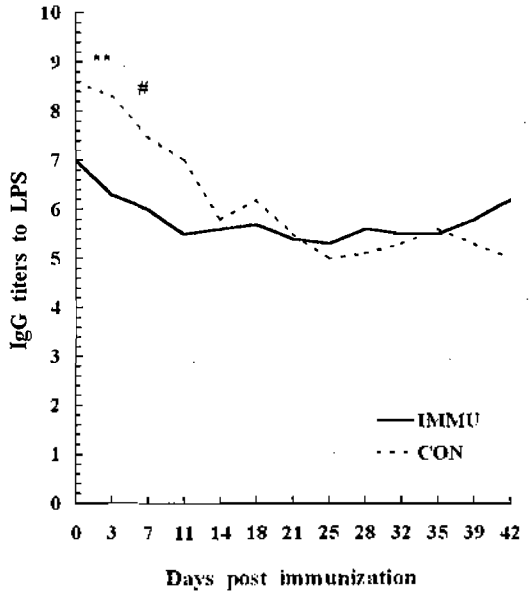

Figure 3. IgG titers to lipopolysaccharide (LPS) in control (CON) and immunization (IMMU) group. SE_{IMMU} = 0.224, SE_{CON} = 0.263; # $p<0.10$, ** $p<0.01$

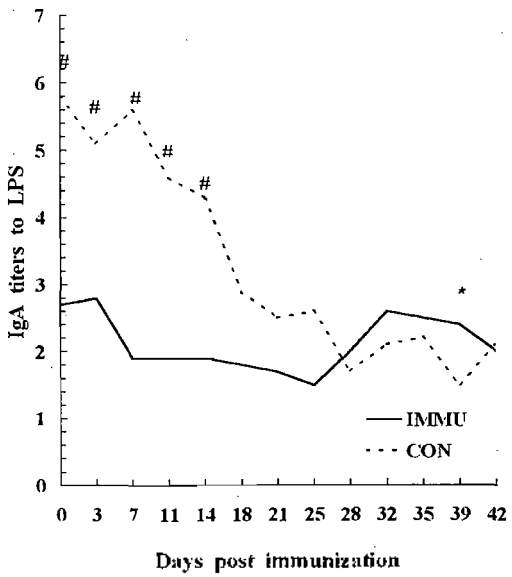

Figure 4. IgA titers to lipopolysaccharide (LPS) in control (CON) and immunization (IMMU) group. SE_{IMMU} = 0.565, SE_{CON} = 0.561; # $p<0.10$, ** $p<0.05$

Table 1. Correlation coefficients between IgG titers to KLH and IgG titers to LPS in immunization (IMMU) group[1]

		IgG to KLH												
		d0	d3	d7	d11	d14	d18	d21	d25	d28	d32	d35	d39	d42
IgG to LPS[2]	d0	-[3]	-	-	-	-	-	-	-	-	-	-	-	-
	d3	-	-	-	-	-	-	-	-	-	-	-	-	-
	d7	-	-	-	-	-	-	-	-	-	-	-	-	-
	d11	-	-	-	-	-	-	-	-	-	-	-	-	-
	d14	-	-	-	-	-	-	-	-	-	-	-	-	-
	d18	-	-	-0.54#	-	-	-	-	-	-	-	-	-	-
	d21	-0.71*	-0.60*	0.61*	0.53#	-	0.62*	0.58*	-	-	-	-	0.55#	-
	d25	-	-0.54#	-	-	-	-	-	-	-	-	-	-	-
	d28	-	-	-	0.62*	0.67*	0.70*	0.67*	0.72**	0.74**	0.68*	0.71*	0.63*	0.68*
	d32	-	-	-	-	-	-	-	-	0.51#	-	-	-	0.53#
	d35	-	-	-	-	-	-	-	-	0.53#	0.51#	0.52#	-	0.54#
	d39	-	-	-	-	-	0.54#	0.58*	0.66*	0.69*	0.68*	0.69*	0.66*	0.70*
	d42	-	-	-	-	-	-	0.52#	0.59*	0.60*	0.60*	0.59*	0.56#	0.59*

[1] Immunized with KLH (Keyhole Limpet Hemocyanin), OA(Ovalbumin) and TT (Tetanus Toxoid); [2] Lipopolysaccharide; [3] Correlation coefficients are not significant; # $p<0.10$; * $p<0.05$; ** $p<0.01$.

Table 2. Correlation coefficients between IgM titers to KLH and IgG titers to LPS in immunization (IMMU) group[1]

		IgM to KLH												
		d0	d3	d7	d11	d14	d18	d21	d25	d28	d32	d35	d39	d42
IgG to LPS[2]	d0	-[3]	-	-	-	-	-	-	-	-	-	-	-	-
	d3	-	0.62*	-	-	-	-	-	-	-	-	-	-	-
	d7	-	-	-	-	-	-	-	-	-	-	-	-	-
	d11	-	-	-	-	-	-	-	-	-	-	-	-	0.50#
	d14	-	-	-	-	-	-	-	-	-	-	-	-	-
	d18	-	-	-	-	-	-	-	-	-	-	-	-	-
	d21	-	-	-	-	-	-	-	-	-	-	-	-	-
	d25	-	-	-	-	-	-	-	-	-	-	-	-	-
	d28	-	-	0.61*	0.57#	-	0.63*	0.56#	0.66*	0.55#	0.63*	0.60*	0.65*	-
	d32	-	-0.52#	0.50#	0.56#	-	0.63*	0.75**	0.75**	0.78**	0.90***	0.92***	0.89***	0.71**
	d35	-	-0.58*	-	0.55#	-	0.59*	0.70*	0.73**	0.87***	0.92***	0.92***	0.83***	0.67*
	d39	-	-	0.53#	0.64*	0.55#	0.65*	0.64*	0.72**	0.78**	0.85***	0.83***	0.72**	0.56#
	d42	-	-	-	0.63*	0.55#	0.66*	0.73**	0.75**	0.80**	0.89***	0.89***	0.75**	0.61*

[1,2,3] See table 1. # $p<0.10$; * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Table 3. Correlation coefficients between IgG titers to LPS and average daily gain (ADG) in immunization (IMMU) group[1]

	IgG to LPS[2]												
	d0	d3	d7	d11	d14	d18	d21	d25	d28	d32	d35	d39	d42
ADG1 (d0 - d7)	-0.24	-0.23	-0.25	-0.59*	-0.44	-0.09	-0.45	-0.02	-0.45	-0.60*	-0.45	-0.36	-0.42
ADG2 (d7 - d14)	-0.31	-0.47	-0.49	-0.61*	-0.56#	-0.14	-0.30	0.11	0.10	-0.28	-0.11	0.09	-0.02
ADG3 (d14 - d21)	0.00	0.00	-0.12	-0.27	-0.15	-0.21	-0.59*	-0.34	-0.31	-0.30	-0.29	-0.22	-0.22
ADG4 (d21 - d28)	0.18	0.08	-0.08	-0.17	-0.42	-0.07	-0.06	-0.77**	0.05	0.10	0.21	0.41	0.39
ADG5 (d28 - d35)	-0.16	0.14	-0.21	-0.22	0.05	-0.13	-0.30	-0.18	-0.53#	-0.65*	-0.68*	-0.73**	-0.74**
ADG6 (d35 - d42)	0.11	-0.33	-0.36	-0.60*	-0.58*	-0.05	-0.29	-0.62*	-0.22	-0.27	-0.21	0.04	-0.12
mean ADG (d0 - d42)	-0.03	-0.15	-0.21	-0.55#	-0.47	-0.17	-0.48	-0.54#	-0.36	-0.45	-0.28	-0.19	-0.27

[1,2] See table 1. # $p<0.10$; * $p<0.05$; ** $p<0.01$.

DISCUSSION

The present study was designed to investigate impacts of humoral immunization of the host animal on the translocation of intestinal bacteria and on performance depression due to immune stimulation induced by translocated enterobacteria. Lipopolysaccharide (LPS) is a component of the cell wall of Gram-negative bacteria and can enter the circulation via the gastrointestinal tract (Wan et al., 1989) under certain conditions, such as gut epithelium damage. Therefore, we have assumed that blood/systemic antibody titers against *E. coli*-derived LPS may be the indicator of the intensity of bacterial translocation, since the blood LPS should be originated from the gut only unless *E. coli* or LPS itself are injected intravascularly or intramuscularly.

Figure 1 shows the immunized piglets mounted typical humoral immune responses to the immunogens (KLH, TT, OA) injected, and the effects of immunization on energy metabolism have been reported

elsewhere (Gentry et al., 1997). As shown in figures 2, 3 and 4, piglets used had already high antibody titers to LPS at the beginning of the experiment. That means enterobacteria or their components, LPS, have already translocated by some mechanisms as suggested by Wells (1990), such as direct transmural migration across the bowed wall and migration into the mesenteric lymph nodes by way of lymphatic and/or vascular channels. There are several reports documenting that facultative bacteria such as *E. coli* and enterococci can translocate across a histologically intact intestinal epithelium (Onderdonk et al., 1974; McConville et al., 1981; Wells et al., 1986). Berg et al. (1988) also reported that intestinal bacterial overgrowth coupled with immunosuppression synergistically promoted levels of bacterial translocation in mice with a histologically normal intestinal tract.

On the other hand, significant ($p<0.10$, 0.05, or 0.01) difference was found in Ig titers to LPS between treatments before the immunization (on d0, piglets were immunized after blood samples were taken), since piglets were not randomized based on anti-LPS Ig titers because the present study was the secondary objective of the experiment. Therefore, anti-LPS responses during the early stage in the present study might be considered as the response to LPS which already translocated and/or existed, rather than the response caused by treatment. Interestingly, however, Ig titers to LPS, which already existed, decreased in both treatments during the first three or four weeks (figures 2, 3 and 4). One reason for the decrease of Ig titers to LPS might be explained by that the piglets used for this study were transported from the conventional environments to the completely disinfected respiration chambers. Nevertheless, the reason still remains unclear.

Thereafter, Ig titers to LPS tended to increase in immunization group, while those in control group continuously decreased (figures 2, 3 and 4). As a result, in spite of significantly higher Ig titers to LPS in CON group on d0, the difference disappeared with time, and moreover IgA titers to LPS became significantly higher in IMMU group ($p<0.05$) on d39 (figure 4). It is seemingly that immunization might affect gut barrier function and facilitate bacterial translocation.

Within the IMMU group, correlation coefficients were calculated between Ig titers to 3 immunogens (KLH, TT, OA) and IgG titers to LPS. The correlations were calculated day by day throughout the observation period, since it may take several days or weeks before immunization could possibly affect the gut barrier and serum antibody levels against LPS as well. In general, correlations between antibody responses to 3 immunogens (KLH, TT, OA) and those to LPS showed very similar tendency, so only the correlations with KLH were presented. As shown in tables 1 and 2, IgG titers to LPS were positively related to IgG and IgM titers to KLH. Those positive correlations might be interpreted in two ways. Firstly, it might be explained by the difference in antibody-producing ability against foreign antigens among individuals. That is to say, the pigs with higher Ig titers to KLH (despite all pigs being immunized with the same amount of KLH in IMMU group) may produce more antibodies against the same amount of LPS existed in the circulation. The second possible explanation is that gut barrier function was more impaired in the pigs with higher Ig titers to KLH, which facilitated the translocation of bacteria including *E. coli* and LPS, and consequently increased Ig titers to LPS. Considering the negative correlation between IgG titers to LPS and average daily gain (table 3), however, the latter interpretation likely to have more possibility.

Table 3 shows how humoral immune response to LPS is related to growth performance of piglets. Generally, average daily gain was negatively related with IgG titers to LPS. That is to say, the growth performance was depressed in pigs which had high antibody responses to LPS. This may be comparable with the result of van Heugten et al. (1994, 1996). They reported that LPS challenge decreased feed intake and daily gain in weanling piglets, though exposure to LPS in the present study was performed not by direct injection to the host but by indirect bacterial translocation from the intestinal tract. Several researchers have also reported that sustained immune responses induced by multiple injections of a variety of immunogens might decrease growth rate and feed intake (Klasing et al., 1987; Klasing and Barnes, 1988; Klasing et al., 1991). And those changes were thought to be mediated, at least in part, by cytokines released from stimulated leukocytes, including interleukin 1 and 6 (Klasing, 1984; McCarthy et al., 1985; Sipe, 1985; Klasing et al., 1987; Klassing, 1988), and tumor necrosis factor (Beutler and Cerami, 1986; Beutler and Cerami, 1988).

Collectively, the present study is likely to reveal some evidences that immunization on the host body might affect gut barrier and facilitate bacterial translocation, based on the differences in the change of antibody responses against *E. coli*-derived LPS between immunized and non-immunized piglets, and on the positive correlations between antibody responses to immunogens injected and those to LPS. And it was clearly demonstrated that growth performance was depressed in piglets with high antibody responses to LPS. Further studies, however, are needed to demonstrate direct evidence of bacterial translocation with the use of nonmetabolizable markers such as lactulose or mannitol (Deitch, 1990).

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