2002

Thermal Environmental Effects and Group Size on Growing Swine Immune Status

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SWINE 2001 - 29

The effects of environmental conditions on the immune system of growing pigs (30-50 kg) were studied using T and B cell proliferation counts. Pigs were exposed to a constant 32°C ambient temperature versus a naturally occurring diurnal temperature variation during hot weather and a constant 10°C vs 21°C ambient temperature during cold weather. In addition, T and B cell proliferation counts were compared for pigs in single, 9 and 18 pigs/pen group sizes. Concancavalin A (ConA), Phytohemagglutinin (PHA) and Staphylococcal enterotoxin B (SEB) mitogens were used to determine T cell activation over time. Pokeweed (PWM), Lipopolysaccharide (LPS) and Salmonella typhimurium mitogen (STM) mitogens were used to determine B cell proliferation over time. ConA tests revealed significant ($P<0.01$) increases in T cell proliferation over time for both temperature treatments during hot weather. No significant differences in B cell proliferation were noted during the hot weather trials. A general decline in T cell activation over time was noted in both temperature treatments during cold weather. Significant ($P<0.01$) reductions in B cell activation were noted for all pigs in the cold weather trials. T and B cell proliferation comparisons for group size at all temperature treatments were non-significant. The objective of this experiment was to find what effects temperature and group size have on the capacity and function of a growing pig's immune system.

(Key words: growing swine, temperature, group size and immune status)

Experimental Procedure

Hot weather treatments consisting of three 28-day replications compared the performance of growing pigs exposed to 1) 21°C plus natural diurnal variations in ambient air temperature and 2) a steady state hot temperature condition of 32°C. These trials were conducted from June 4th to July 9th, July 16th to August 20th and from August 27th to October 1st, 1997 at South Dakota State University's Southeast Experiment Farm near Beresford, SD. Southeast. Pigs were acclimated for one week prior to each 28-day test period at 21°C plus any natural diurnal temperature variations.

Cold weather treatments consisting of three 28-day replications compared the performance of growing pigs exposed to steady state 1) 10°C and 2) 21°C ambient air temperature conditions. The three replications of cold weather trials were conducted from November 18th to December 23rd, 1997, from January 6th to February 10th and from February 27th to March 27th, 1998. Pigs were acclimated for one week prior to each 28-day test period at a steady state temperature of 21°C.

All pigs were weighed and randomly allotted to test pen using weight blocks (Light and Heavy) to reduce within pen weight variation. Allotment of pigs in each test room included two pens, (2.4 x 4.6 m), stocked with 18 pigs with a pen density of 0.62 m^2/pig; two pens (1.2 x 4.6 m) stocked with 9 pigs at 0.62 m^2/pig; and two pens (1.2 x 4.6 m) along the center dividing wall with one pig in each pen (5.5 m^2/pig) for a total of 56 pigs in each trial. The ratio of barrows to gilts in each of the 9 and 18 pigs per group test pens was kept constant (i.e., 9 pig group: 5 barrows: 4 gilts; 18 pig group: 10 barrows: 8 gilts) depending on the number of barrows and gilts delivered to the site.

Phytohemagglutinin (PHA), Concancavalin A (ConA) and Staphylococcal enterotoxin B (SEB) were used to determine T cell activation or proliferation in these studies. To determine the level of B cell activation, Pokeweed (PWM) was used. In addition to PWM, the set of trials during the hot weather period utilized Salmonella typhimurium mitogen (STM). STM was removed from the market before the cold weather tests. Therefore, a switch was made to the more common B cell stimulant, *E.coli* LPS

[1]The authors wish to thank the South Dakota Pork Producer Council for their partial support of this project.

(Lipopolysaccharide) from the O55 strain. All the assays were designed to offer simple probes in the overall capacity of the pig to mount successful immune responses. Blood samples were collected in heparinized vaccutaner tubes on days 1, 14 and 28 of each trial. Ten-milliliter (ml) blood samples were collected by jugular venipuncture from the same 10 pigs in each treatment group. Triplicate wells containing 1 µg/ml of Concanavalin A (ConA), phytohemagglutinin (PHA) and Staphylococcal entertoxin B (SEB) (All from Sigma) were prepared to evaluate T cell proliferation. Triplicate wells containing 5 µg/ml of pokeweed mitogen and 5 µg/ml of Salmonella typhimurium mitogen (STM) or 20 µg/ml of E.*coli* 055 LPS (Lipopolysaccharide) were prepared to evaluate B cell proliferation. Triplicate wells containing only the medium were used as proliferation controls. The plates were incubated 48 hours at 37°C in a humidified, CO_2 incubator. The cells were pulsed for the last six hours of incubation with 0.2 µCi of ^3H thymidine and harvested onto glass fiber filters using a semi-automatic harvester. Filters were counted by liquid scintillation. These counts were then used to calculate the Stimulation Index (SI), which determined the T cell and B cell proliferation of the samples. The Stimulation Index was calculated by the following equation:

SI= mean CPM mitogen stimulated cells/mean CPM control cells for each animal
CPM = Counts per minute

The data from the 2 x 3 x 3 factorial experiment was compiled and analyzed for T cell and B cell differences induced by the thermal treatment, group size and time. Within each experiment the same animals were evaluated initially (day 1), at the mid-point (day 14) of exposure and on termination (day 28) of the experiment. Differences between animals housed in groups of 1 vs 9 vs 18 within a treatment (i.e., high or low temperature) and between treatment and control animals were also evaluated.

Results

Comparisons of T and B cell mitogen responses from pigs exposed to 21°C plus natural diurnal ambient temperature variations and 32°C ambient temperatures at day 0, 14 and 28 are presented in Table 1. Group size and Group size*Time interactions Stimulation Index (SI) are presented in Table 2. These values represent the pooled SI from 30 animals (3 replications) at each time point for pigs raised under a constant hot (32C) and normal conditions (21C+).

Pigs across all group sizes exposed to 21C+ temperatures had significantly (P>0.003) greater ConA response (Table 1) than pigs in the 32C treatment over time, but the increase was greater for pigs in the 21C+ treatment group. When all test pigs from 21C+ and 32C treatments were combined, a significant (P<0.0001) increase in T cell function was observed from day 0 (SI = 30.6) to day 28 (SI = 66.6) in response to ConA. There was no response to ConA when group sizes (Table 2) were evaluated. However, the general trend for all group sizes was an increasing T cell proliferative response to ConA from day 0 to day 28 of the experiment.

The Temperature*Time PHA response (Table 1) of pigs decreased from day 0 to day 28 (P<0.06) in the 21C+ treatment group and increased (P<0.05) from days 0 and 14 to day 28 for pigs exposed to the 32C temperatures. Group size (Table 2) also generated a response (P<0.08) to PHA when all three time periods were averaged. Single penned pigs (SI= 93.0) had a significantly (P<0.05) higher level of response than pigs in the 18 pig per pen group (SI = 64.7). T cell proliferation response using SEB, revealed no significant differences due to temperature or time (Table 1). There were no significant effects of group size on T cell proliferation using SEB (Table 2).

There were no significant B cell responses from PWM when comparing the SI from pigs exposed to the 21C+ and 32C treatments and over the three time periods (Table 1). On day 28, pigs in the 21C treatment had significantly (P<0.05) greater B cell proliferation than pigs in the 32C group as evidenced by STM. The decrease in B cell proliferation from STM between day 0 and day 28 was also significant (P<0.05) for pigs in the 32C room. Group size did not affect B cell proliferation in either temperature treatment (P>0.10).

Comparisons of T and B cell mitogen responses from pigs exposed to 21 and 10°C temperatures from days 0, 14 and 28 are shown in Table 3. T and B cell mitogen response from Group size and Group Size* Time interactions are presented in Table 4. These values represent the pooled SI from 20 pigs (2^{nd} and 3^{rd} replications) at each time point for pigs raised under control (21C) and cold conditions (10C). Data from the first replication was not used because data was only

collected on days 1 and 28 and not on day 14.

There was no significant T cell response (Table 3) to ConA when considering temperature and group size over time for the 21C vs 10C treatments. The PHA test revealed a slight significant ($P<0.06$) response comparing overall means from the 21C to 10C room. When all the pigs were pooled, the SEB tests showed that time had a significant effect ($P<0.002$) with decreasing T cell proliferation from day 1 (SI =51.5) to day 28 (SI=30.4). Considering that there was no significant Time*Temperature interaction, these tests would indicate that T cell proliferation, whether decreasing or increasing, would be similar for pigs raised in either a 21C or 10C environment. The ConA, PHA and SEB tests (Table 4) also revealed that group size did not have a significant effect on increasing or decreasing T cell proliferation over time.

B cell mitogenic activity was generally decreased over time when all pigs were pooled from both temperature treatments. The PWM results showed a significant ($P<0.003$) steady decrease in B cell proliferation from day 1 to day 28 for all pigs over time. Decreasing proliferation from day 1 to days 14 and 28 of B cell mitogens from LPS was also significant ($P<0.01$) over time when all the pigs were pooled. The largest decrease occurred between days 1 and 14. Group size did not significantly affect B cell proliferation. This decreasing B cell trend may represent a more general effect on lymphocyte activation, antigen presentation or cytokine activity in animals.

The data in this experiment showed a generalized decline over time in the proliferative responses to the B and T cell mitogens for pigs in both the 21C and 10C treatments. The animals raised under low temperature conditions showed a progressive reduction over time in lymphocyte responsiveness to all three T cell and both B cell stimulants. However, considerable variability was observed in the proliferative levels of both the control (21C) and the low temperature (10C) between the two replications. Some of the variability may be attributed to a possible outbreak of ileitis during the acclimation and first two weeks of the 3rd replication. Pigs from the third replication in both treatments were mass treated for ileitis with soluble Tylan in addition to the 44 mg/kg of Tylan added to the feed. Pigs in this group were also showing symptoms indicating salmonella. Tissue samples submitted to SDSU Veterinary Diagnostic Lab from two pigs revealed a major stomach ulcer and scar tissue in the lungs. After treatment, it was generally observed by the unit manager that pigs in the 21C treatment appeared to be less affected by the disease problem than the pigs in the 10C treatment and also showed a better response to the medication.

Discussion

Immune system activation can be viewed as beneficial when needed to fight infectious diseases or as negative when unnecessary activation diverts resources from growth and metabolism. The increase in T cell proliferation measured by the ConA lectin provides evidence of immune system activation for pigs exposed to the 21C+ and 32C temperature treatments. The greater T cell proliferation for pigs exposed to the natural diurnal temperature conditions than for the pigs exposed to the constant high temperature of 32°C is of great interest. Pigs in the 21C+ treatment were typically exposed to an average temperature of 25°C with a diurnal variation of +/- 5°C. In conjunction with the varying temperature, these pigs were also continually exposed to pen airflows of 0.4 to 0.5 m/s. This airflow was the result of having the ventilation system set at approximately 70% of maximum capacity when ambient air temperatures reached 22°C and at 100% full capacity when ambient temperatures reached 23°C. These settings are typical for ventilation systems in swine production facilities. It appears that the combination of varying temperatures and sustained draft had greater impact on T cell proliferation increases when compared to pigs at a constant high temperature with no draft.

From these studies, there is evidence that cold stress has an effect on the overall capacity of B cells to respond in pigs. A pronounced and consistent generalized depression of B cell mitogenesis under cold stress conditions was observed. Cold stress caused a decrease in T cell proliferative capacity over time in our experiments as well. This was in contrast with the animals under heat stress. It appears that the 28-day cold stress exposure induced a generalized depression of the immune responses, suggesting a broad effect on immune competence of cold stressed animals. The cold stress conditions used in these trials had a broad spectrum effect on the immune function of the pigs. The pigs had reduced responsive capacity in all tests measured over time. This suggests that some underlying immunological mechanism was significantly affected by the cold stress conditions, one that

influences all the specific immune response capacity of the pigs. Further study is required to define the basis of this defect.

Group size did not have any significant effect on immune system activation in any of the temperature treatments. This suggests the stresses associated with group size affect the immune system equally regardless of temperature.

Implications

The greater increase in T cell proliferation for pigs exposed to naturally occurring diurnal temperature change with the associated higher air speeds at pig level over the constant high temperature should generate some concerns on how ventilation systems are managed during warm weather. It may not be in the best interest of lighter weight pigs to have the ventilation system at maximum capacity, thus creating a high airflow over the pigs. The general trend of decreasing T cell and B cell proliferation for pigs exposed to temperatures below the thermoneutral zone may impede the pig's ability to fight off infectious disease.

All the assays were designed to offer simple probes in the overall capacity of the pig to mount successful immune responses. Further studies can now follow which examine the critical functions of immune responses in thermally stressed pig.

TABLE 1. TIME AND TEMPERATURE STIMULATION INDEX (SI) COMPARISONS FOR HOT WEATHER

	Temperature		Time		Temp x Time		P Values		
Item	21 C+[a]	32C[b]			21C+	32C	Temp	Time	Temp x Time
No. of Pigs	30	30		60	30	30			
T-Cell:			Day						
CONA[c]	44.7 (6.8)[h]	44.3 (6.8)	0:	30.6[j]	26.8[j]	34.5[m]			
			14:	36.3 (7.1)[k]	29.6[k]	43.1 (8.2)	NS[l]	P<0.0001	P<0.003
			28:	66.6[jk]	77.7[jkl]	55.4[lm]			
PHA[d]	73.5 (15.3)	81.3 (15.3)	0:	83.2	87.1	79.4			
			14:	68.6 (15.5)	69.6	67.6[k] (17.1)	NS	NS	P<0.06
			28:	80.4	63.7[j]	97.1[jk]			
SEB[e]	64.6 (6.2)	67.0 (6.2)	0:	59.5	62.0	57.0			
			14:	67.3 (6.5)	63.0	71.3 (9.2)	NS	NS	NS
			28:	70.6	68.0	72.7			
B-Cell									
PWM[f]	73.0 (6.9)	61.4 (6.9)	0:	65.3	65.8	64.6			
			14:	69.4 (8.0)	71.2	67.5 (10.5)	NS	NS	NS
			28:	67.0	81.8	52.1			
STM[g]	49.7 (4.9)	40.0 (4.9)	0:	48.7	45.5	51.9[k]			
			14:	43.8 (5.6)	49.6	38.0 (7.3)	P< 0.09	NS	P<0.08
			28:	42.0	54.0[j]	30.0[jk]			

[a]21C+ = 21°C plus natural diurnal variation
[b]32C = 32°C constant
[c]Concancavalin A
[d]Phytohemagglutinin
[e]Staphylococcal entertoxin B
[f]Pokeweed mitogen
[g]Salmonella typhimurium mitogen
[h]= Standard Error
[l]Non-significant (P>0.1)
[jklm]Significantly different means (P<0.05)

TABLE 3. TIME AND TEMPERATURE SI COMPARISONS FOR COLD WEATHER

Item	Temperature		Time		Temp x Time		P Values		
	21[a]	10C[b]			21C	10C	Temp	Time	Temp x Time
No. of Pigs	20	20	40		20	20			
T-Cell:			Day						
CONA[c]	28.4 (24.9)	38.9 (24.9)	0:	40.5	49.2	31.9			
			14:	31.4 (24.9)	31.0	31.8 (25.4)	NS[l]	NS	NS
			28:	29.0	36.5	21.4			
PHA[d]	26.6 (6.7)	14.4 (6.7)	0:	21.5	22.6	20.5			
			14:	18.1 (6.8)	24.5	11.6 (7.9)	P<0.06	NS	NS
			28:	21.9	32.5	11.3			
SEB[e]	44.8 (24.7)	36.4 (24.7)	0:	51.5[j]	44.1	58.9			
			14:	39.9[j] (24.6)	42.2	37.5 (23.8)	NS	P<0.002	NS
			28:	30.4[j]	22.9	38.0			
B-Cell									
PWM[f]	50.0 (25.0)	56.5 (25.0)	0:	70.5[j]	62.0	79.0			
			14:	50.3[j] (25.2)	47.7	52.9 (26.1)	NS	P<0.003	NS
			28:	39.0[j]	40.3	37.7			
LPS[g]	24.4 (14.4)	14.2 (14.4)	0:	31.7[j]	41.3	22.2			
			14:	11.1[j] (14.6)	14.7	7.4 (15.5)	NS	P<0.01	NS
			28:	15.8[j]	18.4	13.1			

[a] 21C = 21°C constant
[b] 10C = 10°C constant
[c] Concancavalin A
[d] Phytohemagglutinin
[e] Staphylococcal entertoxin B
[f] Pokeweed mitogen
[g] Lipopolysaccharide
[h] = Standard Error
[l] Non-significant (P>0.1)
[jklm] Significantly different means (P<0.05)

TABLE 2. TIME AND GROUP SIZE SI COMPARISONS (LEAST SQUARE MEANS) FOR HOT TREATMENTS

Item	1[a]	9	18	P Values GS[i]	P Values GS x Time
No. of pigs					
T-Cell:	12	24	24		
CONA[b]					
0[g]	35.4 (10.3)[h]	27.6 (8.6)	28.9 (8.6)		
14	42.1 (10.3)	29.8 (8.6)	37.1 (8.6)	NS[j]	NS
28	71.1 (10.3)	70.7 (8.6)	57.9 (8.6)		
$\bar{x}$	49.5 (7.7)	42.7 (7.0)	41.3 (7.0)		
PHA[c]					
0	112.2 (20.1)	78.0 (17.6)	59.5 (17.7)		
14	83.8 (20.1)	65.6 (17.6)	56.5 (17.7)	P< 0.08[k]	NS
28	83.0 (20.1)	80.1 (17.6)	78.1 (17.7)		
$\bar{x}$	93.0 (16.6)	74.6 (15.7)	64.7 (15.7)		
SEB[d]					
0	75.6 (13.2)	52.0 (10.2)	51.0 (10.0)		
14	79.6 (13.2)	66.0 (10.1)	56.2 (10.2)	NS	NS
28	74.9 (13.2)	71.5 (10.1)	65.5 (10.2)		
$\bar{x}$	76.7 (8.6)	63.2 (7.1)	57.6 (7.1)		
B-Cell					
PWM[e]					
0	62.1 (15.1)	61.8 (11.4)	71.9 (11.1)		
14	82.5 (15.1)	59.4 (11.1)	66.2 (11.3)	NS	NS
28	68.1 (15.1)	68.6 (11.1)	64.2 (11.3)		
$\bar{x}$	70.9 (9.3)	63.3 (7.2)	67.4 (7.2)		
STM[f]					
0	66.4 (10.4)	35.0 (7.9)	44.7 (7.7)		
14	47.8 (10.4)	40.7 (7.7)	42.9 (7.8)	NS	NS
28	43.1 (10.4)	40.6 (7.7)	42.4 (7.8)		
$\bar{x}$	52.4 (6.5)	38.8 (5.1)	43.3 (5.1)		

[a]Group size (pigs/pen) 1, 9 or 18
[b]Concancavalin A
[c]Phytohemagglutinin
[d]Staphylococcal entertoxin B
[e]Pokeweed mitogen
[f]Salmonella typhimurium mitogen
[g]= Time, days
[h]= Standard Error
[i]Group size
[j]NS = non-significant (P> 0.1)
[k]1 (93.0) vs. 18 (64.7) P< 0.05

TABLE 4. TIME AND GROUP SIZE SI COMPARISONS (LEAST SQUARE MEANS) FOR COLD TREATMENTS

Item	1[a]	9	18	P Values GS[i]	P Values GS x Time
No. of pigs					
T-Cell:	8	16	16		
CONA[b]					
0[g]	36.4 (26.5)[h]	38.7 (26.6)	46.4 (25.6)		
14	28.3 (26.5)	28.2 (25.7)	37.9 (25.6)	NS[j]	NS
28	31.6 (26.5)	24.4 (25.7)	30.9 (25.6)		
$\bar{x}$	32.1 (25.4)	30.4 (25.1)	38.4 (25.6)		
PHA[c]					
0	18.6 (9.9)	12.4 (8.3)	33.6 (8.3)		
14	8.5 (9.9)	26.6 (8.3)	23.1 (8.3)	NS	NS
28	25.4 (9.9)	20.5 (8.3)	19.8 (8.3)		
$\bar{x}$	17.5 (7.7)	18.5 (7.1)	25.5 (7.1)		
SEB[d]					
0	58.5 (26.2)	48.6 (25.5)	47.4 (25.5)		
14	36.9 (26.2)	36.7 (25.5)	46.0 (25.5)	NS	NS
28	26.1 (26.2)	32.5 (25.5)	32.5 (25.5)		
$\bar{x}$	40.5 (25.2)	39.2 (25.0)	42.0 (25.0)		
B-Cell					
PWM[e]					
0	77.7 (28.0)	75.8 (26.3)	58.1 (26.3)		
14	41.8 (28.0)	42.4 (26.3)	66.5 (26.3)	NS	NS
28	31.5 (28.0)	40.7 (26.3)	44.8 (26.3)		
$\bar{x}$	50.4 (25.8)	53.0 (25.2)	56.5 (25.2)		
LPS[f]					
0	51.7 (17.5)	16.4 (15.8)	27.1 (15.8)		
14	6.9 (17.5)	8.7 (15.8)	17.6 (15.8)	NS	NS
28	20.6 (17.5)	13.3 (15.8)	13.4 (15.8)		
$\bar{x}$	26.4 (15.2)	12.9 (14.8)	19.4 (14.6)		

[a]Group size (pigs/pen) 1, 9 or 18
[b]Concancavalin A
[c]Phytohemagglutinin
[d]Staphylococcal entertoxin B
[e]Pokeweed mitogen
[f]Lipopolysaccharide
[g]= Time, days
[h]= Standard Error
[i]Group size
[j]NS = non-significant (P> 0.1)