Reevaluation of the Necessity of Iron Injection to Newborn Piglets

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ABSTRACT : The growth and immune responses to endotoxin lipopolysaccharide (LPS) challenge (20 μg/kg) of piglets with and without a iron dextran injection (Fe, 200 mg/head) two days after birth are compared. Sixty-four newborn piglets from eight litters were allocated randomly to one of four treatments. The control received no iron dextran and only saline (Sal) injection on the second and fifteenth day after birth (Sal-Sal). The remaining three groups received Fe-Sal, Sal-LPS, Fe-LPS treatments respectively. On fifteen days of age, blood samples of piglets were taken at 0 h, 1 h, 2 h and 4 d after saline or LPS injection to determine immune functions and blood characteristics. The trial terminated when the pig reached 56 days and the average daily gain of piglets was then measured. Daily gain, serum immunoglobulin G (IgG) concentration and red blood cell counts did not vary significantly among the four groups at any measuring times. Serum tumor necrosis factor-α (TNF-α) concentration increased sharply 1 h after LPS injection. However, iron injection did not change TNF-α concentration responds to LPS injection. White blood cell counts of two LPS injection groups were significantly lowered 1 h following the injection. In contrast, serum lactoferrin concentration had increased significantly 1 and 2 h post-injection. Furthermore, iron injection produced no further effects on these two criteria. Iron injection increased the hemoglobin (Hb) concentration of piglets at any measuring time, and LPS injection lowered Hb concentration. In conclusion, a 200 mg/head of iron dextran injection on the second day after birth increased Hb concentration, had no detrimental effect on the immune responses and growth of piglets. Moreover, if creep feed (175 mg Fe/kg feed) is provided from d 7 after birth, the Fe-injection does not contribute to overall performance of piglets and may not be a necessity in practice. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 1 : 79-83)***

Key Words : Iron Injection, Growth, Immune, Piglets

INTRODUCTION

The iron a newborn piglet stores will be insufficient three to seven days after birth (Thoren-Tolling and Jonsson, 1977). In addition to rendering piglets anemia, this iron deficiency depresses their growth and may cause diarrhea (Lemacher and Bostedt, 1994). Therefore, piglets are generally injected intramuscularly with a 200 mg iron dextran two or three days after birth to prevent anemia and improve growth (Parsons, 1979; Pollmann et al., 1983). However, evidence now strongly suggests that iron injection may be hazardous. That is, either the Fenton reaction or the iron-catalyzed Hovber-Weiss reaction produces hydroxyl radicals, which are responsible for the damaging effect of iron. Furthermore, clinical conditions associated with iron excess in humans or animals may increase the risk of infection (Oppenheimer, 1989; Ward et al., 1996; Walter et al., 1997; Patruta and Horl, 1999). Some studies reveal that due to the bacteria release siderophores, which accelerate the iron transport mechanism (Mitra, et al., 1988), although iron supplementation increases iron available to the host, it favors potential microbial invaders more. Moreover, both iron excess and deficiency have been proven to enhance bacterial endotoxin (lipopolysaccharide, LPS) toxicity and impair phagocytes and also increase macrophages tumor necrosis factor-α (TNF-α) production (Omara et al., 1994).

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Received April 25, 2001; Accepted August 13, 2001

Pig mortality is a great concern during nursery, and respiratory and digestive diseases are two main factors of deaths. The likelihood of an iron injection lowering the immune function of nursing pigs requires investigation. This study attempts to re-evaluate the need for iron injection in newborn piglets based on their growth and immune responses.

MATERIALS AND METHODS

Animals and experimental procedure

Sixty-four newborn piglets from eight litters of Landrace × Yorkshire × Duroc crossbred were randomly assigned (by litter basis) into four treatments: TRT 1 : Control group, piglets received only 1 ml saline (Sal) intramuscular injection on day (d) 2 and 15 after birth (Sal-Sal); TRT 2 : iron injection group, a dose of 200 mg/head on d 2, and 1 ml saline on d 15 (Fe-Sal); TRT 3 : LPS injection group, 1 ml saline on d 2 and a 20 μg/kg of LPS (Serotype 026:B6, Sigma Chemical, St. Louis, MO) on d 15 (Sal-LPS); and TRT 4 : injected with iron dextran 200 mg/head on d 2 and LPS on d 15 (Fe-LPS). A creep feed (table 1) was administered *ad libitum* to all piglets starting from d 7 and replaced by a starter diet (table 1) from d 28 (weaning) to d 56. Animals had free access to water throughout the study. On d 15 blood samples (6 ml) was withdrawn from the anterior Vena Cava of all piglets prior to either LPS or Sal injection, as well as 1 h, 2 h and 4 d following either injection. A small portion (1 ml) of blood was poured into a tube that contained heparin to determine

Table 1. Diet formulas and nutrient compositions of creep feed and starter diet

Ingredients, %	Creep feed	Starter diet
Corn, dent yellow, grain	50.9	63.7
Soybean meal, solv, 44%	4.0	10.0
Soy protein concentrate, 64%	8.7	9.0
Skim milk, dried	6.0	5.0
Whey, dried	15.0	-
Fish meal, anchovy, 65%	2.6	6.0
Tallow	2.5	3.0
Blood plasma, spray dried	7.0	-
Enzymes (Entose)	0.1	0.4
Limestone, pulverized	0.80	0.78
Calcium phosphate (monocalcium)	1.63	1.56
Salt	0.20	0.26
Vitamin premix[a]	0.10	0.10
Mineral premix[b]	0.15	0.10
L-Lysine	0.10	-
L-Methionine	0.22	0.10
Calculated nutrient composition		
Crude protein, %	23.0	21.0
Digestible energy, kcal/kg	3,610	3,520
Calcium, %	1.00	0.95
Phosphorus, %	0.9	0.8
Iron, mg/kg	175	128

a Each kg of vitamin premix contained : vitamin A, 6,000,000 IU; vitamin D_3, 800,000 IU; vitamin E, 20 g; vitamin B_2, 4 g; vitamin B_{12}, 0.02 g; pantothenic acid 12 g; niacin, 40 g; folic acid, 0.4 g; biotin, 0.1 g; choline chloride, 50 g.

b Each kg of trace mineral premix contained : Cu, 4.5 g; Fe, 70 g; Mn, 15 g; Zn, 80 g; I, 0.3 g.

the red blood cell (RBC) and white blood cell (WBC) counts, as well as hemoglobin (Hb) concentrations. The remaining blood samples were collected into other tubes and stored for 1 h at room temperature for coagulation. Blood clots were then centrifuged (2,500×g for 20 min at 4°C) and serum was saved and preserved at -20°C for subsequent measurements of serum immunoglobulin G (IgG), TNF-α and lactoferrin concentration (Lf). The investigation ended and the average daily gain (ADG) was measured when piglets reached 56 d of age.

Analysis of serum composition and blood cell characteristics

According to the procedure that Hankins et al. (1992) described, total serum IgG concentration was detected by using an ELISA kit for porcine IgG (ICN Pharmaceuticals, Inc. Ohio, USA). An ELISA porcine TNF-α kit (Endogen, Cambridge, MA) was used to measure total serum TNF-α. Serum samples were assayed in duplicate at either 1:1 or 1:10 dilution. The assay was sensitive to 10 pg/ml of TNF-α and had an intraassay CV of 9.48%. Serum Lf concentration was also determined by ELISA developed by Chu et al. (1993) using rabbit anti-porcine Lf IgG (100 ng/well) and mouse anti-porcine Lf antiserum. Blood cells counts and Hb concentration were determined by using Hematologic Analyzer 7290 (Roche Diagonostic System Co., USA).

Statistical analysis

The effects of treatments on ADG, immune responses and blood characteristics were analyzed using the GLM procedure of SAS (1992) as a repeated two-way ANOVA. When ANOVA revealed a significant effect, differences among treatment means were tested using Duncan's New Multiple Range Test (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Table 2 summarize the effects of iron and LPS challenge on ADG, and on blood characteristics of piglets. ADG of piglets did not differ significantly among the four treatments from d 0 to 14 and d 14 to 56 after birth. Evidently, iron injection did not improve ADG of piglets significantly. Iron administered either by injection or orally can enhance piglet growth rate (Zimmermann et al., 1959). This enhancement, however, may not necessarily show; Furugouri (1972) employed similar treatments to that of Zimmermann et al. (1959) and noted no improvements in piglet growth rate. The effect of iron injection on growth rate of suckling piglets seems to depend on the blood iron concentration of the pig, the amount of milk and creep diet taken after birth. In this study, anemia was not observed in control piglets, and LPS injection (TRT 3 and 4) did not abate their ADG throughout the whole experiment. It appears that the supply of creep feed on d 7 provided an amount of iron that was sufficient for growth and that the iron injection on d 2 has no additional benefit in promoting growth.

It has been demonstrated that high dose LPS injection exceeding 100 μg/kg caused a significant reduction in weight gain and/ or feed intake in pigs (Wright et al., 2000). In this study, only one injection with a comparatively low LPS dose (20 μg LPS/kg) was administered 15 d after birth. This dosage emulates the study of Spurlock et al. (1997) in which a 25 μg/kg BW of LPS was injected intramuscularly. Due to the rapid elimination rate of LPS (Van Deventer et al., 1990; Yoshida et al., 1995), a 20 μg/kg BW injection did not affect piglet growth rate, and yet was sufficient to challenge immune-system.

Excess of free intracellular iron results in oxidative stress (Kar and Chakraborti, 1999; Patruta and Horl, 1999; Touati, 2000). This excess may undermine acquired immunity (Gutteridge, 1995; Halliwell, 1996; Wisemann, 1996; Knight, 2000). Furthermore, it is believed that iron supplementation may actually weaken resistance towards

Table 2. Effect of iron injection and LPS injection on growth and immune responses of piglets.

Items	Treatments				SEM
	Sal-Sal (n=16)	Fe-Sal (n=16)	Sal-LPS (n=16)	Fe-LPS (n=16)	
Daily gain, g					
0-14 d	145^{B}	156^{A}	153^{A}	151^{A}	8
14-56 d	216^{A}	241^{A}	232^{A}	240^{A}	12
Serum IgG, ng/ml					
Pre-LPS/ Sal	47	45	46	45	3
1 h post-LPS/ Sal	44	47	47	49	3
2 h post-LPS/ Sal	49	45	53	47	3
4 d post-LPS/ Sal	41	39	47	46	3
Serum TNF-α, pg/ml					
Pre-LPS/ Sal	69^{B}	76^{B}	71^{B}	59^{B}	5
1 h post-LPS/ Sal	$626^{A,b}$	$557^{A,b}$	2471^{Aa}	$2918^{A,a}$	242
2 h post-LPS/ Sal	58^{Bc}	67^{Bbc}	157^{Bab}	163^{Ba}	16
4 d post-LPS/ Sal	54^{B}	60^{B}	54^{B}	55^{B}	3
Serum Lf, ng/ml					
Pre-LPS/ Sal	55.7^{A}	66.5	46.1^{B}	$55.7^{B,C}$	6.9
1 h post-LPS/ Sal	$48.5^{A,B,b}$	59.5^{ab}	99.1^{Aa}	$99.0^{A,B,a}$	7.6
2 h post-LPS/ Sal	$30.2^{A,B,b}$	54.6^{b}	134.5^{Aa}	$134.8^{A,a}$	8.0
4 d post-LPS/ Sal	18.7^{B}	44.1	24.7^{B}	44.5^{C}	4.9

^{A,B,C} Means of the same column with the different superscript were significantly different ($p<0.05$).
^{a,b,c} Means of the same row with the different superscript were significantly different ($p<0.05$).

infection (Mitra et al., 1988; Ward et al., 1996; Walter et al., 1997), and the poor ability of lymphocytes to sequester excess iron may explain the immune system abnormalities in overloaded patients (Walker and Walker, 2000). However, this study reveals that a 200 mg iron dextran injection 2 d after birth did not retard the immune responses of newborn piglets. The concentrations of IgG of TRT 2 were not lower than the control group, revealing that Fe treatment hardly affected the serum IgG concentration of healthy piglets. This is also true in piglets under LPS challenge since IgG concentration profile was similar between TRT 3 and TRT 4 (table 2). These results indicate that Fe treatment exerts no effect on serum IgG concentration whether LPS challenge exists or not. Bruininx et al. (2000) reported that in newborn piglets, a double intramuscular injection (day 3 and 21 after birth) of 200 mg Fe did not affect their growth or total IgG response to Keyhole limpet hemocyanin and ovalbumin. It can be concluded that 200 mg iron dextran injection has no obvious detrimental effect on piglets.

When bacterial endotoxin is present, macrophages excrete TNF-α which controls the activity of different WBC and thus regulates and optimizes the immune and inflammatory responses (Webel et al., 1997). It is known that TNF-α also regulates the acute phase response, induces the production of other immunoregulatory cytokines, and activates T cells, B cells, NK cells, and neutrophils (Sharon et al., 1999). Peripheral LPS injection elevates TNF-α concentration in the plasma of weaned pigs (Webel et al., 1997; Wright et al., 2000). In this study, LPS injection caused a sharp increment of TNF-α concentrations of TRT 3 and 4 ($p<0.05$) to a level similar at 1 h post injection, despite Fe treatment. Following saline injection, the concentration of TNF-α were also significantly elevated ($p<0.05$), but to a similar extent in saline and iron-injected pigs. Notably, LPS injection and the injection stress itself, not the iron treatment, influenced TNF-α concentration (table 2). Serum Lf concentration was increased after 2 h following LPS injection on TRT 3 and 4 to a similar level, and saline treatments had no effect. Iron injection did not alter Lf response (table 2). Zagulski et al. (1998) proved that in the defense system of mice, lactoferrin primarily kills bacteria, and that its plasma levels increased significantly 120 min after the start of LPS infusion.

Blood characteristics of piglets receiving different treatments are shown in table 3. Iron concentration dropped significantly 2 h after LPS injection. The experimental results presented herein agree with those reported by Gutteberg et al. (1989). Roth et al. (1997) demonstrated that when LPS preincubated with Fe, the binding of Fe to LPS reduced LPS potency and decreased LPS-induced lethality in mice. However in other investigations, the separate injection of Fe and LPS, without preincubation, did not

Table 3. Effects of iron injection and LPS challenge on blood characteristics of piglets

Items	Treatments				SEM
	Sal-Sal (n=16)	Fe-Sal (n=16)	Sal-LPS (n=16)	Fe-LPS (n=16)	
Serum Fe, μg/dl					
Pre-LPS/Sal	83^{b}	168^{a}	59^{b}	174^{a}	8
1 h post-LPS/Sal	73^{b}	163^{a}	50^{b}	145^{a}	10
2 h post-LPS/Sal	83^{b}	183^{a}	46^{b}	165^{a}	9
4 d post-LPS/Sal	76^{b}	177^{a}	82^{b}	176^{a}	9
White blood cells, $\times 10^{3}$/μl					
Pre-LPS/ Sal	10.1	9.6	8.5^{A,B}	7.7^{B}	0.6
1 h post-LPS/Sal	9.1^{a}	9.5^{a}	4.2^{C,b}	4.6^{C,b}	0.6
2 h post-LPS/Sal	8.7^{a}	7.8^{a}	5.0^{B,C,a,b}	3.4^{C,b}	0.6
4 d post-LPS/Sal	10.4	11.9	10.2^{A}	10.9^{A}	0.4
Red blood cell, $\times 10^{6}$/μl					
Pre-LPS/ Sal	7.3	6.2	6.8	6.6	0.4
1 h post-LPS/Sal	6.7	6.8	5.6	7.1	0.3
2 h post-LPS/Sal	6.9	6.9	5.7	6.9	0.3
4 d post-LPS/Sal	6.1	5.9	5.5	6.9	0.2
Hemoglobin, g/μl					
Pre-LPS/ Sal	11.7^{A,a,b}	11.9^{a,b}	9.6^{b}	14.7^{a}	0.7
1 h post-LPS/Sal	10.9^{ABb,c}	12.9^{a,b}	8.9^{c}	14.2^{a}	0.6
2 h post-LPS/Sal	11.4^{Aa,b}	13.4^{a}	8.4^{b}	13.8^{a}	0.5
4 d post-LPS/Sal	8.1^{B,b}	11.5^{a,b}	8.4^{b}	15.1^{a}	0.8

^{A,B,C} Means of the same column with the different superscript were significantly different ($p<0.05$).
^{a,b,c} Means of the same row with the different superscript were significantly different ($p<0.05$).

result in less lethality than with LPS alone (Prigal et al., 1973). This finding infers that LPS detoxification requires direct contact between the two agents. In this study, Fe and LPS were injected separately, which may explain why the Fe and LPS group (TRT 4) had no better immune functions than the group of LPS alone.

Herein WBC counts decreased significantly ($p<0.05$) 1h after LPS injection (TRT 3 and 4), which then restored gradually. This revealed that the damaging effect of LPS on WBC only lasted several hours and Fe treatment (TRT 2 and 4) did not affect WBC changes after LPS injection. However, no impairment to these WBC was revealed under a 200 mg/head of iron dextran injection. The absence of such impairment of the WBC indicated that for the newborn piglets a 200 mg iron dextran injection was not an overload. Blood RBC counts were similar among treatment groups at any period of blood sampling. Conversely, blood Hb concentration of the two Fe treatment groups (TRT 2 and 4) revealed higher than the other two groups (TRT 1 and 3, table 3) were. This corresponded to the serum iron concentration in our piglets. Under LPS injection, serum Hb concentrations tend to be lower, but if iron is previously injected, Hb concentration (TRT 4) will remain higher than TRT 2. Other reports contain similar experimental results (Kay et al., 1980; Lemacher and Bostedt, 1995; Egeli et al., 1998).

Iron overload is associated with an increased susceptibility to certain infections (Oppenheimer, 1989; Patruta and Horl, 1999). In human patients iron enhances suppressor T-cell (CD8) numbers and activity, decreases the proliferate capacity of helper T cells (CD4) with an increase in CD8/CD4 ratios, and impairs cytotoxic T cells generation (Walker and Walker, 2000). In the present study, a 200 mg injection of iron dextran in 2 d old piglets did not depress their immune function, although it slightly but insignificantly increased their Hb concentration and weight gain. However, since early creep feeding (containing 175 mg Fe/kg feed and 7 d old onward) is sufficiently to sustain a growth rate, which is comparable to those that received Fe treatment. Thus, iron injection, may not be a necessity. It is premature to suggest that iron dextran injection can be abandoned insofar, this common practice nevertheless, deserves a further evaluation from aspects of management and economics.

ACKNOWLEDGEMENTS

The authors would like to thank the National Science Council of the Republic of China for financially supporting this research (NSC 88-2313-B-059-021). Dr. H. C. Wu is appreciated for his lactoferrin analysis and the chemical analysis assistance that C. Lu, J. H. Chen provided is

appreciated.

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