Effect of Threonine Addition to a Low Protein Diet on IgG Levels in Body Fluid of First-Litter Sows and Their Piglets

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ABSTRACT : First-litter gilts were used to determine how different dietary treatments during gestation affect the reproductive performance of gilts and immunity development of their piglets. Twenty-two crossbred Landrace×Yorkshire gilts were randomly assigned to three dietary treatments. Following conception, the gilts were fed experimental diets until farrowing occurred. The diet for treatment 1 was low protein diet (8% CP), treatment 2 had an additional supplement of 0.14% threonine that was added to the low protein diet, and treatment 3 was a control diet containing 12% CP. During gestation, net body weight gain of sows in treatment group 2 was higher than in treatment group 1 ($p=0.075$). However, during lactation there was no difference between all treatments groups on body weight loss and their live piglets at birth. Although milk IgG between treatments did not differ, treatment groups 2 and 3 were slightly higher than treatment group 1 was. Plasma IgG concentrations in piglets were however equal within all treatment groups at birth and at 7 days of age, at 21 days of age, it was higher in treatment group 1 than it was in the other two groups ($p<0.01$). Threonine supplementation to a low protein diet during gestation slightly increases milk IgG of sows. It is beneficial for piglets to acquire more passive immunity, but a suppressive effect was also noted on the endogenous IgG synthesis in piglets. A gestation diet of 8% CP for gilts can stimulate immuno-system of her piglets. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 8 : 1157-1163)*

Key Words : Immunoglobulin G, Piglets, Pregnancy Diets, Sows, Threonine

INTRODUCTION

Dietary protein and content balance of amino acids are two major concerns of the diet for breeding pigs and considerable experimental evidence indicates that nutrition exerts a great influence on reproductive efficiency as well as immune system in sows (Den Hartog et al., 1994). Recently, amino acid nutrition and the importance of threonine for immunoproteins synthesis in colostrum and milk to maintain health and intestinal integrity of piglets has been emphasized with respect to immunocompetency (Han and Lee, 2000). It has been reported that dietary protein level affects milk production in the sow, but fails to affect milk amino acid composition, neither on plasma immunoglobulin concentration in gestating and lactating sows nor the resistance of their piglets against antigens (King et al., 1993). However, in a sorghum-based diet supplemented with lysine and threonine, Cuaron et al. (1984) demonstrated that lysine is the first limiting amino acid for gravid gilts, but threonine is the first limiting amino acid which maintains circulating IgG concentrations. Therefore, it can be hypothesized that dietary threonine and not protein may affect the humoral immunoglobulins in pregnant sows, as well as in piglets considerably through colostrum or milk.

There is a complex correlation among nutrition, disease prevention and the establishment of immunocompetence in newborn piglets (Kelley and Easter, 1991). The vast difference in threonine, leucine, valine, phenylalanine, and immunoglobulins concentrations between colostrum and milk further exacerbates the complexity (Beacon and Bowland, 1951). Related research benefits pig husbandry as well as the understanding of nutrition in general. The purpose of this experiment was to determine the effects of a low protein diet supplemented with threonine on plasma and whey IgG concentrations in sows during late gestation and lactation, and as well as plasma IgG concentrations in piglets.

MATERIALS AND METHODS

Animals and diets

Twenty-two crossbred Landrace×Yorkshire gilts (above 120 kg) were selected and they were successfully mated after showing estrus and subsequently allocated to three dietary treatments (table 1). Treatment 1 was a low protein diet (8% CP), which contained corn, soybean meal and wheat bran and was supplemented with lysine, tryptophan, and methionine at 0.37%, 0.06%, and 0.09% respectively. In treatment 2, an additional 0.14% L-threonine supplement ed the low protein diet. Treatment 3 consisted of a normal diet, which contained 12% CP.

Feeding and management

After mating and treatment allocation, all gilts were maintained and fed in individual cages. Prior to day 80 of gestation the feeding level was 1.8 kg, which was increased from day 81 to farrowing to 2.4 kg. Hence, the average daily feed intake was 1.98 kg. All animals were fed twice

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Table 1. Composition of experimental diets

	Treatment 1 8% protein basal diet	Treatment 2 8% protein diet +0.14% threonine	Treatment 3 12% protein diet	Lactation diet[f]
Ingredient(%)				
Corn, dent yellow	90.61	90.47	79.44	72.81
Soybean meal, solvent	1.10	1.10	12.80	18.63
Wheat bran	4.50	4.50	4.50	6.00
Dicalcium phosphate	1.79	1.79	1.79	1.42
Limestone, polished	0.78	0.78	0.78	0.54
Salt	0.50	0.50	0.50	0.40
Vitamin premix[a]	0.10	0.10	0.10	0.10
Mineral premix[b]	0.10	0.10	0.10	0.10
Threonine		0.14		
Other amino acids supplement[c]	0.52	0.52		
Analyzed composition				
Crude protein, %	7.94 (8.0)[e]	8.01(8.0)	11.93(12.0)	15.00
Digestible energy, MJ/kg (calculated value)	14.1	14.1	14.1	14.0
Essential amino acids, %[d]				
Lysine	0.53 (0.55)	0.53 (0.55)	0.54 (0.55)	0.76
Tryptophan	0.09 (0.13)	0.09 (0.13)	0.10 (0.13)	0.20
Threonine	0.31 (0.33)	0.45 (0.47)	0.41 (0.47)	0.60
Methionine+cystine	0.25 (0.44)	0.36 (0.44)	0.31 (0.44)	0.54
Arginine	0.40	0.36	0.70	0.96
Histidine	0.21	0.19	0.29	0.42
Leucine	0.95	0.83	1.24	1.55
Isoleucine	0.27	0.24	0.44	0.66
Phenylalanine	0.31	0.31	0.50	0.76
Valine	0.37	0.34	0.56	0.77

[a] Supplied the following vitamins per kilogram of diet: vitamin A 5000 IU; vitamin D_3 600 IU; vitamin E 20 IU; vitamin K_3 2 mg; vitamin B_2 2mg; vitamin B_{12} 0.02 mg; niacin 30 mg; pantothenic acid 10 mg; biotin 0.01 mg.

[b] Supplied the following minerals per kilogram of diet: Zn 80 mg; Cu 6 mg; Fe 80 mg; Mn 10 mg; Se 0.1 mg; I 0.2 mg; choline chloride 60 mg.

[c] Containing lysine 0.37%, tryptophan 0.06%, and methionine 0.09%.

[d] Total amino acid. [e] Value in parentheses represents calculated value.

[f] The composition of lactation diet listed [included] in calculated value.

daily and had free access to water throughout the study. During the last 3 to 4 days of gestation, the gilts were moved to farrowing cages. All sows were fed with the same lactation diet after farrowing (table 1). The sows were weighed at mating, 110 day of gestation, farrowing, and weaning. Piglets were weighed within 24 h of birth, litter size (n=9) was standardized at three days of age, and piglets were weaned at 28 days of age.

Samples collection and analysis

Blood samples (10 ml) were collected from sows by vena cava puncture at d 80 and 110 of gestation, at farrowing, and at d 7 and 21 of lactation. All blood samples were collected in a tubes that contained EDTA. After farrowing, three piglets with the heaviest body weight were selected from each litter and blood samples (2 ml) were collected via vena cava puncture at 0 (after suckling colostrum for about 6 h), 7, and 21 days of age. Blood samples were centrifuged and plasma was collected and stored in -20°C until IgG analysis.

Milk samples were obtained after an intravenous injection of oxytocin (10 units). Sows were milked manually to collect approximately 30 ml milk from the first and second anterior teats soon after farrowing as well as on d 7 and 21 of lactation. To remove the fat layer, milk samples were centrifuged at 17,100 g for 30 min at 4°C. Then to obtain whey (middle layer of the tube) they were centrifuged at 198,000 g for 90 min and at 20°C. The whey was stored at -20°C until IgG analysis.

Measurement of porcine immunoglobulin G

Porcine plasma and whey IgG concentrations were

analyzed by an ELISA assay described by Hankins et al. (1992). A 96-well microtiter plate (Corning catalog no. 25801-96) was initially coated with 50 μl of a 1:1000 dilution of rabbit anti-porcine IgG (H+L) (catalog no. 61-9100, Zymed Laboratories, Inc.). The plate was then incubated at 37°C for 1 h washed three times with a rinse buffer (500 ml of 10 times condensed PBS buffer and 3 ml of Tween-20 dissolved in 4.5 l of H_2O, pH 7.4), and blotted dry. Subsequently, 50 μl blocking buffer containing 1% BSA was added to each well, which was followed by an incubation at 37°C for 1 h and three rinse buffer washes. Porcine IgG stock (catalog no. I-4381, Sigma Chemical) was diluted in PBS buffer (NaCl, 0.137 mol; KCl, 0.027 mol; Na_2HPO_4, 0.005 mol; KH_2PO_4, 0.001 mol; dissolved in 1 l of dH_2O) to generate a standard IgG curve. Plasma and whey samples were diluted to 200000× with PBS buffer and 50 μl was added to the wells in triplicate. Microtiter plates were then incubated at 37°C for 1 h and washed three times with a rinse buffer. A 50 μl of a 1:1000 dilution of alkaline phosphatase conjugated rabbit anti-porcine IgG (H+L) (catalog no. 61-9122, Zymed Laboratories, Inc.) in alkaline phosphatase buffer (Tris-HCl, pH 8.8, 0.05 M, NaCl 0.15 M; EDTA, pH 8.01 mM, Tween-20 0.05%; and BSA, 0.1%) was added and incubated at 37℃ for 1 h followed by three washes. Finally, 50 μl of alkaline phosphatase substrate (two 5-mg tablets of phosphatase substrate [catalog no. N-9389, Sigma Chemical] in 10 ml of alkaline phosphatase substrate buffer [Na_2CO_3, 0.022 mol; $NaHCO_3$, 0.028 mol; $MgCl_2.6H_2O$, 0.002 mol; dissolved in 1 l of dH_2O]) was added to all wells and incubated at room temperature until color development occurred. Fifty μl of 3 M NaOH was then added to stop the reaction. Plates were read at 405 nm (Bio-tek EL-340 Bio Kinetics Reader) by subtracting blank value (average value of the zero standards).

Statistical analysis

Data were analyzed as a complete randomized design using the GLM procedure of SAS (1988). Treatments were included as the main effect in the model to test all dependent variables with individual sows used as the experimental unit. The least significant difference test was employed to separate means, where the probability of $p<0.05$ was considered significant and at $p<0.10$ it had tendency. Although, the randomized means of body weight at conception between treatments differed slightly, covariance analysis was employed to render them equal to further compare the adjusted means.

RESULTS AND DISCUSSION

Table 2 lists the effects of a low protein diet that was supplemented with threonine during gestation on feed intake, body weight of first-litter gilts, and performance of piglets. Table 3 lists plasma and whey IgG of gilts and plasma IgG levels in piglets.

At conception, body weight in treatment groups 2 and 3 was slightly higher than that of treatment group 1 was, though the difference was not statistically significant. On day 110 of gestation, body weight in treatment group 2 or group 3 was higher than in treatment group, even so there was no difference between treatment groups 2 and 3. During gestation net body weight gain in treatment group 2 surpassed that of treatment group 1 slightly ($p<0.10$). During lactation, feed intake, weaning body weight, body weight loss, farrowing backfat, weaning backfat, and backfat loss between treatments were similar among the treatment groups. After farrowing, the sows of treatment group 2 were heavier than those in treatment group 1 were, based on adjusted weight ($p<0.05$).

Speer (1990) indicated that if a gilt weighting 120 kg at mating, and gained 25 kg maternal weight and 20 kg products of conception during pregnancy, a daily ration of 1.8 kg with 90% true digestibility, which contained 142.4 g of protein would meet the pregnancy and moderate growth requirement. However, the diet had to contain adequate levels of essential amino acids. In this study an 8% CP diet with 1.98 kg daily allowance provided a protein level that exceeded the one Speer (1990) recommended. Although the isoleucine level of groups 1 and 2 was slightly lower than that of group 3 was, they satisfied the estimated requirement (Speer et al., 1990). Notably, the contents of other essential amino acids also met or exceeded the levels recommended by Speer (1990) or NRC (1988). That is, nutrients provided in all treatment groups satisfied the daily requirements of gilts during gestation.

Chang et al. (2000) concluded that a 60% dietary threonine : lysine ratio (1.33% lysine) for growing gilts resulted in a better growth performance as well as higher nutrient utilization and lower BUN concentration. King and Brown (1993) indicated that a first-litter gilt weighting 100-135 kg has the greatest nitrogen retention, which is similar to a finishing pig. Williams et al. (1993) also indicated that the optimal ratio of threonine and lysine in a finishing diet is 0.68. Herein, in groups 1, 2, and 3 these ratios were 0.59, 0.85, and 0.76, respectively, and therefore, the insufficiency of threonine content in diet 1 explained the lower weight gain during gestation. When diets 1 and 2 were compared, the supplementation with 0.14% threonine in the second increased the weight gain during gestation, although body weight loss during lactation was similar to the other two groups. The dietary protein level in diet 2 was lower than that in diet 3, but the threonine level in both diets (table 1) was similar and this similarity attributes to the comparable body condition during gestation and lactation of these two

Table 2. Effect of low protein diet supplemented with threonine during gestation on body condition of first-litter gilts and performance of piglets

Performance of traits	Treatment 1 8% protein basal diet	Treatment 2 8% protein diet +0.14% threonine	Treatment 3 12% protein control diet
Number of gilts	8	8	6
Feed intake, kg/d			
Gestation	1.98	1.98	1.98
Lactation	4.1±0.4	3.8±0.4	3.8±0.6
Body wt, kg			
At conception	129.7±6.9	144.4±8.7	146.9±8.7
(Adjusted)*	(138.5)	(138.5)	(138.5)
At d 110 gestation	176.6±4.0^{c}	190.2±4.9^{d}	181.1±4.9^{cd}
Gain during gestation	38.1±4.0^{c}	51.6±4.9^{d}	42.6±4.9^{cd}
After farrowing	157.8±4.2^{a}	172.8±5.2^{b}	165.3±5.8^{ab}
At weaning	146.0±6.5	159.2±8.0	155.7±9.0
Loss during lactation	11.8±3.6	13.6±4.4	9.6±4.9
Back fat, cm			
At farrowing	1.9±0.1	1.7±0.1	1.7±0.1
At weaning	1.8±0.1	1.6±0.1	1.6±0.1
Loss during lactation	0.1±0.06	0.1±0.05	0.1±0.07
Performance of piglets			
Live born/litter	9.6±1.3	10.5±1.3	9.0±1.5
Live litter wt at birth, kg	14.5±1.9	14.9±1.9	12.0±2.2
Live birth weight, kg	1.5±0.1	1.5±0.1	1.4±0.1
Litter size at 3 days of age after standardizing	9.6±1.2	8.8±2.9	9.0±2.6

^{a,b,c,d} Means in the same row with different superscript are different (^{a,b} $p<0.05$ and ^{c,d} $p<0.10$).

* Implies adjusted by covariance analysis to the same body weight at conception (listed in parentheses).

treatments. Evidently, threonine was the limiting factor for the body growth of sows during gestation.

Regarding piglet performance, there was no difference between treatments. Mahan and Mangan (1975) reported that there is no relationship between protein intake, protein source during gestation, and subsequent litter size and birth weight of pigs. However, when protein or amino acid supply is deficient during pregnancy, maternal gain is affected primarily. Unless sows are very severe protein restricted with protein-free diets or with very low supplies, the birth weight of piglets would not be reduced (Pond et al., 1992; Pluske et al., 1995). In the present experiment, the dietary protein levels among treatments differed significantly, though the levels were sufficient and the performances characteristic of piglets, as expected, were not affected. Obviously, if nutrients supplied do not meet gestation requirements maternal protein and fat reserves would be consumed, to ensure fetal demands are met, especially during late gestation. Alternately, feed intake during gestation is an important factor that affects milk production (Dourmad, 1991; Klindt et al., 1999; Weldon et al., 1994) that subsequently determines growth preference during nursing.

Plasma IgG concentrations of sows in the three treatment groups were similar throughout the study (table 3). Plasma IgG concentrations in all treatment groups were similar during late gestation, but dropped significantly ($p<0.01$) at farrowing. Notably, the plasma IgG in all treatment groups increased during lactation. This corresponds with the finding of Klobasa et al. (1986) that plasma IgG in sows decline greatly at farrowing. This obvious decline could be due to the mammary absorption of IgG. The plasma appears to serve as a metabolic pool from which amino acids and proteins (such as IgG) are withdrawn and are utilized by the mammary gland to meet the demands for milk secretion (Cuaron et al., 1984).

Despite the high threonine content in the immunoglobulins (Smith and Greene, 1947; Beacom and Bowland, 1951; Tenenhouse and Deutsch, 1966), our experiment showed that if threonine levels in the gestation diet were higher than those recommended by NRC (1988) or Speer (1990), additional threonine or protein in the feed did not affect the homeostasis of sow plasma IgG concentration. Haye et al. (1981) indicated that the protein content of sow's food ration did not affect the transfer of maternal antibody to piglets. Corley et al. (1983) also found

that when a low protein diet is administered during gestation, which has a higher protein diet during lactation the synthesis of sow antibodies is unaffected.

Table 3 lists colostral IgG concentrations in the 3 treatment groups at farrowing. The concentration in treatment group 1 was lower than that in treatment groups 2 and 3, however, due to a great individual variation, they did not differ statistically. Our experimental result was consistent with that of Klobasa et al. (1986), which indicated that there were large colostral IgG variations in individual sows and particularly during the first 12 h of lactation. Colostral IgG is transferred primarily from plasma, not synthesized by mammary gland cells (Bourne and Curtis, 1973). Mammary glands can extract amino acids and IgG from plasma, which they then secrete into colostrum, thus determining plasma IgG of sows after farrowing and during early lactation.

Although slightly lower in treatment group 1 (1.20 mg/ml) than in treatment group 3 (1.49 mg/ml) ($p=0.089$), whey IgG concentration on d 7 of lactation did not differ between treatments on d 21 of lactation. This suggests that threonine or crude protein levels during pregnancy have limited affect on the whey IgG concentration after 3 weeks of lactation. The effect of supplementing threonine in the low crude protein diet on whey IgG concentration requires further study. In fact, since IgA in milk plays an essential role in protecting piglets from gut pathogens (Tonks, 1995), both IgG and IgA levels in milk should be investigated, as a slight decrease in IgA or IgG may have an unexpected consequence.

While colostral IgG in treatment group 1 was somewhat lower than in treatment groups 2 and 3 at farrowing, plasma IgG concentrations in their piglets at birth were not different after colostrum sucking. There is no endogenous immunoglobulin in piglets at birth (Curtis and Bourne, 1971), therefore, among the various immuno- globulins, colostral IgG is especially essential. Payne and Marsh (1962) discovered, that the γ-globulin absorption in the small intestine of piglets is in accordance with the all-or-none phenomenon. That is, once the epithelial cells of intestinal mucosa have been fully occupied with γ-globulin or perhaps any soluble protein, no further absorption occurs. In our experiment, the all-or-none phenomenon might have made the epithelial cells of intestinal piglet mucosa stop immunoglobulin absorption during the first few hours after birth. Therefore, despite the different colostral IgG contents between the treatment groups, the plasma IgG levels in piglets were equal. Klobasa et al. (1986) also indicated that the failure to correlate IgG concentrations between colostrum and piglet plasma might result from the difference of milk production of sows. Plasma IgG concentrations in one-week-old piglets will maintain almost the same level as within 6 h of birth, which was roughly 18 mg/ml. Furthermore, all groups presented the same experimental results. Notably, whey IgG concentration in sows within treatment group 1 was lower at farrowing and at d 7 of lactation. However plasma IgG concentration in their piglets at 21d of age was higher ($p<0.01$) than in those

Table 3. Effect of low protein diet supplemented with threonine during gestation on the IgG concentration of first-litter gilts and their piglets

	Treatment 1 8% protein basal diet	Treatment 2 8% protein diet +0.14% threonine	Treatment 3 12% protein diet
Number of gilts	8	8	6
Gilt plasma IgG (mg/ml)			
Gestation d 80	32.2±1.3	31.3±1.2	32.5±1.5
Gestation d 110	31.1±3.0	31.1±3.4	33.9±3.4
Farrowing	17.2±2.7*	16.9±2.7*	21.3±3.0*
Lactation d 7	29.4±1.9	29.5±2.0	31.6±2.2
Lactation d 21	25.1±1.6	22.9±1.6	22.8±1.8
Gilt whey IgG (mg/ml)			
Farrowing	34.6±7.1	44.6±6.0	44.7±7.1
Lactation d 7	1.2±0.1[c]	1.3±0.1[cd]	1.5±0.1[d]
Lactation d 21	1.0±0.1	0.9±0.1	1.1±0.1
Piglet plasma IgG (mg/ml)[b]			
Birth	18.4±1.2	18.5±1.2	18.1±1.3
7 d of age	18.2±1.3	18.6±1.5	17.0±1.8
21 d of age	9.3±0.6[a]	6.9±0.6[b]	6.4±0.8[b]

* Differ significantly ($p<0.01$) when compared to values of d 80 and d 110 of gestation.

[a,b,c,d] Means in the same row with different superscript are different ([a,b] $p<0.05$ and [c,d] $p<0.1$).

from the other two treatment groups. While whey IgG in sows of treatment groups 2 and 3 were similar at farrowing and on d 7 of lactation, their piglets plasma IgG concentrations were also similar at 21 d of age.

In piglets, the half-life of IgG is approximately 14 days (Curtis and Bourne, 1973; Tizard, 1982) and the passively acquired IgG in plasma decreases apparently at 3 weeks of age. Piglets primarily synthesize their own plasma immunoglobulins afterwards (Klobasa et al., 1986). Henry and Jerne (1968) noted that the passively acquired IgG and IgM suppresses the initial immunoglobulin synthesis in piglets. Klobasa et al. (1981) also showed that if piglets suck less milk IgG, the initial IgG synthesis suppression will be lower. Klobasa et al. (1986) further demonstrated that piglets, which passively acquire more IgG will suppress more their IgG synthesis. This decrease in plasma IgG levels also existed in our piglets of treatment groups 2 and 3 at 21 d of age.

In summary, our experimental results showed that gilts fed a corn-soybean diet containing 8% crude protein, which was supplemented with main essential amino acids during gestation, were nearly identical in all aspects to those fed 12% crude protein diet except a slightly but insignificantly lower milk IgG. Supplementation with threonine in a 8% protein diet during gestation increased the milk IgG concentration to a level comparable to that of 12% protein diet and might have increased the maternal IgG supply with piglets in early lactation. It would be beneficial for newborn piglets to acquire more passive immunity for defense against the environmental pathogen. However, if piglets passively acquired more colostral IgG, suppressive or delaying effects might occur on the endogenous IgG synthesis in piglets at 3 weeks of age. The 8% protein diet, therefore is advantageous to stimulate immun system of piglets and hence, has a more substantial value than gestation diet of 12% protein to gilts. Further studies are required to clarify how the IgG synthesis of suckling affects growth of piglets before an overall value of adopting a gestation diet of 8% protein for gilts can be assessed.

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