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Author	Kwangwook Kim[1#], Byeonghyeon Kim[2#], Hyunjin Kyoung[3#], Yanhong Liu[1], Joy M. Campbell[4], Minho Song[3*] and Peng Ji[5*]
Affiliation	[1] Department of Animal Science, University of California, Davis, CA 95616, United States of America [2] Animal Nutrition & Physiology Team, National Institute of Animal Science, Rural Development Administration, Wanju 55365, Korea [3] Division of Animal and Dairy Science, Chungnam National University, Daejeon 34134, Korea [4] APC, Inc., Ankeny, IA 50021, United States of America [5] Department of Nutrition, University of California, Davis, CA 95616, United States of America
ORCID (for more information, please visit https://orcid.org)	Kwangwook Kim (https://orcid.org/0000-0001-5854-6047) Byeonghyeon Kim (https://orcid.org/0000-0003-4651-6857) Hyunjin Kyoung (https://orcid.org/0000-0001-5742-5374) Yanhong Liu (https://orcid.org/0000-0001-7727-4796) Joy M. Campbell (https://orcid.org/0000-0003-2499-3644) Minho Song (https://orcid.org/0000-0002-4515-5212) Peng Ji (https://orcid.org/0000-0002-7447-5688)
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Ethics approval and consent to participate	This experimental protocol for this research was reviewed and approved by the Institutional Animal Care and Use Committee of Chungnam National University, Daejeon, Korea (approval # CNU-00611).

CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Minho Song
Email address – this is where your proofs will be sent	mhsong@cnu.ac.kr
Secondary Email address	mhsong6@gmail.com
Address	Division of Animal and Dairy Science, Chungnam National University, Daejeon 34134, Republic of Korea
Cell phone number	+82-10-9254-0931
Office phone number	+82-42-821-5776
Fax number	+82-42-825-9754

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Peng Ji
Email address – this is where your proofs will be sent	penji@ucdavis.edu
Secondary Email address	
Address	Department of Nutrition, University of California, Davis, CA 95616, United States of America
Cell phone number	
Office phone number	+1-530-752-6469
Fax number	

Abstract

The study was conducted to evaluate the effects of spray-dried plasma (SDP) supplementation during late gestation and lactation on productive performance and immune responses of sows and their litters. Twelve sows (227.78 ± 2.16 kg average body weight; 2.0 average parity) were randomly allotted to two dietary treatments: a basal diet (CON) and the basal diet supplemented with 1% SDP. Sows were fed experimental diets from d 30 before farrowing to weaning of their piglets. Blood samples were collected from sows on d 1, 3, and 7 of lactation and from two randomly selected nursing pigs per litter on d 3 and 7 after birth, and d 1, 3, and 7 after weaning. Productive performance and immune responses of sows and their piglets were measured. There was a trend of less body weight loss in sows supplemented with SDP ($p < 0.10$) during the lactation period and a trend of greater ($p < 0.10$) average daily gain in SDP piglets compared to those in the CON group. Sows in the SDP group tended to have lower ($p < 0.10$) serum concentrations of tumor necrosis factor-α (TNF-α), transforming growth factor-β1 (TGF-β1), and cortisol on d 3 and lower serum concentration of TNF-α on d 7 compared with sows in CON group. In comparison with CON piglets, piglets from SDP sows tended to have lower ($p < 0.10$) serum concentrations of TNF-α, TGF-β1, and cortisol on d 7 after birth, lower ($p < 0.10$) serum TNF-α and C-reactive protein on d 3 and 7 after weaning, and greater ($p < 0.10$) average daily gain after weaning. Moreover, weaned pigs from sows fed SDP had significantly lower ($p < 0.05$) serum concentrations of cortisol and TGF-β1 on d 3 and 7 postweaning, respectively, than CON piglets. In conclusion, spray-dried plasma supplementation in sow diets from late gestation to weaning improved the productive performance of sows and their offspring; the beneficial effects of SDP may be mediated in part through modulation of immune responses of both sows and piglets.

Keywords: spray-dried plasma, lactating sows, productive performance, immune responses, weaned pigs

Introduction

Reproductive performance and health status of sows are economically important factors to the profit of the swine industry. However, numerous environmental risk factors, such as disease and seasonal variation, can affect the reproductive performance of sows and herd productivity [1]. Therefore, various functional dietary supplements or feed additives were investigated for their potential benefits in enhancing the production, health and longevity of sows [2].

Spray-dried plasma (SDP) is a protein-rich feed additive in swine diets with well-balanced amino acid composition and diverse bioactive components, including immunoglobulins, peptides, glycoproteins, and others [3]. It has also been reported that SDP is superior to soybean meal and other plant-derived protein sources with respect to the contents of essential amino acids [4]. SDP is extensively used in nursery pig diet due to its advantages of improvements in health status. Previous studies have shown that supplementation of SDP improved growth performance [5,6], intestinal barrier function [7,8], and immune responses [9,10] of weaned pigs. More prominent positive effects are often observed when SDP is supplemented in diets for younger pigs that have immature immune system or pigs in the unsanitary environment [11], suggesting an immunomodulatory function of SDP. Such immunomodulatory and other beneficial effects of SDP are believed due in part to its high concentration of immunoglobulins [12,13]. Although the precise mechanism is yet unclear, there are several proposed modes of action of SDP including 1) direct effects on the barrier functions of the gastrointestinal tract [14] 2) regulation of gut-associated lymphoid tissue [15], 3) systemic effects on the respiratory [16] and reproductive systems [17]. In addition, SDP products have been suggested as a potential alternative to antibiotics based on their promising beneficial effects on pigs [14].

Supplementation of SDP products in sow diet during gestation or lactation was shown to improve productivity of sow and growth of their offspring. For instance, supplementing 0.5% SDP in gestation diet (from d 28 post-breeding until d 1 after farrowing) of sows increased body weight and average daily gain of litters at weaning [18], which was ascribed to the improved lactation performance in response to SDP supplementation. Moreover, a lower rate of preweaning mortality and greater weaning weight of piglets were reported for multiparous sows fed a diet containing 0.5% of SDP during lactating [19]. A study by Crenshaw et al. [20] also reported that supplementation of 0.5% SDP in lactation diets improved average body weight of weaned pigs and subsequent farrowing rate of sows, while postweaning sow mortality was reduced. However, information on the growth performance and immune responses of nursing and weaned pigs from sows fed SDP during late gestation and lactation period are still limited. Therefore, the objective of this experiment was to investigate not only the effects of SDP supplementation in sow diets on productive performance and immune responses of sows in lactation period but also their litters.

Materials and Methods

Experimental protocol was reviewed and approved by the Animal Care and Use Committee at the Chungnam National University, Daejeon, Republic of Korea (Protocol # CNU-00611). The experiment was performed at the animal research facility at the Chungnam National University.

Animals, housing, experimental design, and diet

A total of twelve lactating sows (Yorkshire × Landrace; 227.78 ± 2.16 kg average body weight; 2.0 average parity) were stratified by parity and randomly assigned to one of the two dietary treatments on d 84 of gestation (6 sows/treatment). Sows were individually housed in farrowing crates since d 109 of gestation and had free access to water. Sows were fed either a basal diet (control, CON) or the basal diet supplemented with 1% (as-fed basis) SDP. The SDP used in this experiment was produced by APC Inc. (Ankeny, IA, USA). All diets met the current estimates for nutrient requirements of sows (Table 1) [21]. Sows were restricted fed the experimental diets (3.0 kg/day) from d 84 of gestation until farrowing, and then fed the experimental diets on an ad libitum basis from until weaning of their piglets.

Litters were weaned at 27 d of age (6.09 ± 0.26 kg average body weight) and each litter was allotted to individual nursery pen. Weaned pigs were subjected to a 3-phase feeding program and fed the phase 1 nursery diet at week 1, phase 2 diet at week 2 and phase 3 diet from week 3 to 6 postweaning. Weaned pigs had free access to water and diets throughout the experiment.

Sample collection and measurements

Lactating sows and their litters' body weight were measured on the day of farrowing and weaning. Body weight change of sows and average daily gain of piglets during the lactation period were calculated. The feed intake for each sow was recorded during lactation period to calculate the average daily feed intake. Measurements of sow backfat depth was performed on P2 position (65 mm down the left side from the spine at the same level as the last rib curve) using a real-time ultrasound scanner (Anyscan BF, SongKang GLC Co., Gyeonggi-do, Republic of

Korea) on day of farrowing and weaning. The number of stillborn and liveborn piglets and weaned piglets were measured to calculate productive performance.

Blood samples were collected from each sow on d 1, 3, and 7 of lactation and two randomly selected piglets (1 barrow and 1 gilt) per litter on d 3 and 7 after birth and d 1, 3 and 7 after weaning using vacutainers containing clot-activator or ethylenediaminetetraacetic acid (EDTA) to harvest whole blood or serum, respectively. Serum samples were collected from whole blood after centrifugation (3,000 rpm for 15 min at 4℃) and kept at -20℃ until analysis.

Measurements of white blood cell counts, serum cytokines, acute phase protein, cortisol, and Immunoglobulins

Whole blood samples were analyzed by an automatic hematology analyzer (scil Vet abc hematology analyzer, scil animal care company, F-67120 Altorf, France) for total white blood cell (WBC) counts. The serum concentrations of tumor necrosis factor-α (TNF-α; R&D Systems, Inc., Minneapolis, MN, USA), transforming growth factor- β1 (TGF-β1; R&D Systems, Inc., Minneapolis, MN, USA), C-reactive protein (Abnova Corp., Taipei City, Taiwan), cortisol (Cusabio, Wuhan, China), and immunoglobulin G, M, and A (IgG, IgM, and IgA; Abnova Corp., Taipei City, Taiwan) were measured using porcine-specific enzyme-linked immunosorbent assay (ELISA) kits by following the instruction manufacturers' instructions.

Statistical analyses

All data were analyzed using the PROC GLM procedure of SAS (SAS Inst. Inc., Carry, NC, USA) in a completely randomized design with the sow or their litter as an experimental unit, respectively. The model for productive performance and immune responses of sows and their litters included the fixed effect of dietary treatment and sow or litter as random terms; Parity of sows was used as a covariate in the model. Statistical significance and tendency were considered at $p < 0.05$ and $0.05 \leq p < 0.10$, respectively.

Results and discussion

Sows supplemented with 1% SDP tended to have less ($p < 0.10$) body weight losses and improved ($p < 0.10$) piglet average daily gain during the time of lactation than those fed CON; however, there were no differences in other productive performances between the groups (Table 2). Sows mobilize body reserves (protein and fat) to support lactation, therefore body weight loss is commonly observed during lactation. However, excessive loss of body weight during lactation can compromise reproductive performance of sows in the subsequent pregnancy, including reduced rate of pregnancy and lower survival rates of embryos [22,23]. Therefore, appropriate feeding practices, such as high-energy rations and nutrient-rich feeds, are required to minimize lactation weight loss [24,25]. Previous research reported that supplementation of 0.25% SDP in lactation diets tended to reduce body weight losses of sows and significantly improved average litter weight at weaning [26]. In agreement with this study, results from the current study showed that dietary supplementation of 1% SDP in late gestation and lactation is effective in reducing weight loss of lactating sows and improving the growth of nursing piglets. Taken altogether, SDP may mitigate body weight loss through enhancing nutrient utilization and lactation performance of sows and thus enhance the growth of their litters.

Compared with CON sows, addition of 1% SDP tended to reduce ($p < 0.10$) serum TNF-α, TGF-β1, and cortisol concentrations on d 3 and serum TNF-α concentrations on d 7 after farrowing (Table 3). No difference was observed in the white blood cell counts and serum C-reactive protein throughout the experiments for sow. Parturition is the most stressful event of the reproduction cycle of sows, while improper pre- and peripartal management and nutrition may lead to chronic stress that has deleterious effects on immune functions [27,28]. As a result, these stress-related alterations reflect immunodeficiency, which may contribute to reproductive failure or death of sows and might adversely affect the performance of their litters [29]. It was reported previously that dietary supplementation of 1 or 8% SDP reduced serum concentrations of inflammation- and stress-related mediators (TNF-α, C-reactive protein, and cortisol) and increased concentrations of anti-inflammatory cytokine (TGF-β1) in the uterine of pregnant mice that suffered from transportation stress [17]. In addition, pregnant mice that received 8% SDP had lower pro-inflammatory cytokines in uterine mucosa and placenta and had reduced lethargic response after LPS challenge [30]. These observations suggest that SDP could attenuate inflammation and enhance immune competence in pregnant mice. Moreover, it was also reported that SDP maintained gut barrier function of weaned pigs [7] and rats [31] in a state of inflammatory status, respectively. Thus, in the present study, the reduced body weight change of sows and greater average daily gain of their litters by SDP supplementation may result from more robust immune functions of sows in response to farrowing and lactation stresses.

Piglets from sows supplemented with SDP tended to have lower ($p < 0.10$) serum concentrations of TNF-α, TGF-β1, and cortisol on d 7 of lactation (Table 4). However, no differences were found in serum immunoglobulins of nursing piglets during lactation (Table 5). After weaning, pigs from sows supplemented with SDP tended to have greater average daily gain ($p < 0.10$) compared with CON piglets (Table 6). This result was supported by inflammatory and stress markers in serum. On d 3, weaned pigs from sows supplemented with SDP had reduced ($p < 0.05$) serum cortisol concentrations, tended to have reduced ($p < 0.10$) serum TNF-α concentration than those from sows in CON group (Table 7). On d 7, serum concentrations of TGF-β1 were decreased ($p < 0.05$) and tended to have reduced ($p < 0.10$) C-reactive protein concentrations in weaned pigs from sows fed SDP compared with those from sows fed CON. In order to acquire passive immunity, newborn piglets have to consume a sufficient volume of colostrum which contains high energy sources and immunoglobulins [32]. Previous studies reported that body weight and body conditions of sows during gestation are important factors that affect the quality and quantity of colostrum and milk production [33–35]. In the current study, increased piglet average daily gain at weaning and improved immune responses of nursing and weaned pigs could have been due to improved health status of sows fed SDP. Moreover, the possible modes of action include, but are not limited to 1) increase the growth rate of piglets from sows supplemented with SDP may have been due to increased production of colostrum and milk and thus increased consumption of colostrum and milk by suckling pigs; 2) sows fed SDP may have produced higher concentrations of immunoglobulins in colostrum and milk, however, the amounts of immunoglobulins contained in sow colostrum or milk were not analyzed, nor was colostrum or milk production of sows measured. Future research is needed to elucidate the mechanisms of improved immune responses in pigs from sows supplemented with SDP.

Conclusion

In conclusion, supplementation of spray-dried plasma during late gestation and lactating sows' diets improved productive performance and immune responses of lactating sows and their litters.

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Table 1. Ingredient composition of experimental diets (as-fed basis)

Item[1]	Gestation diet		Lactation diet	
	CON	SDP	CON	SDP
Ingredients (%)				
Corn	75.82	76.72	65.54	66.53
Soybean meal	21.30	19.40	31.81	29.82
SDP[2]	-	1.00	-	1.00
Limestone	0.90	0.90	0.85	0.85
MDCP	1.58	1.58	1.40	1.40
Vitamin premix[3]	0.20	0.20	0.20	0.20
Mineral premix[4]	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00
Calculated chemical composition				
ME (Mcal/kg)	3.32	3.32	3.43	3.43
CP (%)	15.86	15.82	19.76	19.72
Crude fat (%)	3.09	3.05	2.86	2.80
Crude fiber (%)	2.97	3.00	3.33	3.36
NDF (%)	8.71	8.78	10.78	10.81
ADF (%)	4.18	4.20	4.63	4.65
Calcium (%)	0.77	0.77	0.75	0.75
Phosphorus (%)	0.64	0.65	0.65	0.65
Lysine (%)	0.74	0.76	1.02	1.02
Methionine (%)	0.25	0.25	0.30	0.30
Threonine (%)	0.58	0.60	0.74	0.76
Tryptophan (%)	0.16	0.16	0.22	0.22

[1]CON, control sow diet based on corn and soybean meal; SDP, CON + 1% spray-dried plasma; MDCP, monodicalcium phosphate; ME, metabolizable energy; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber.

[2]Spray-dried plasma (APC Inc., Ankeny, IA).

[3]Provided per kilogram of diet: vitamin A, 10,000 IU; vitamin D_3, 2,000 IU; vitamin E, 48 IU; vitamin K_3, 1.5 mg; riboflavin, 6 mg; niacin, 40 mg; D-pantothenic acid, 17 mg; biotin, 0.2 mg; folic acid, 2 mg; choline, 166 mg; vitamin B_6, 2 mg; and vitamin B_{12}, 28 μg.

[4]Provided per kilogram of diet: Fe, 90 mg from iron sulfate; Cu, 15 mg from copper sulfate; Zn, 50 mg from zinc oxide; Mn, 54 mg from manganese oxide; I, 0.99 mg from potassium iodide; Se, 0.25 mg from sodium selenite.

Table 2. Productive performance of lactating sows fed diets supplemented with spray-dried plasma[1]

Item[2]	Dietary treatments		SEM	*p*-value
	CON	SDP		
Parity	2.1	2.0	0.30	0.733
Lactation days (d)	28.00	26.16	0.76	0.118
Initial BW on d 1 after farrowing (kg)	226.14	229.42	3.12	0.471
Final BW at weaning (kg)	208.80	218.61	3.73	0.092
Sow BW change (kg)	-17.34	-10.81	2.45	0.081
Total feed intake (kg)	183.83	180.48	4.07	0.530
ADFI (kg)	6.57	6.89	0.11	0.114
Initial backfat depth (mm)	20.50	20.75	1.88	0.789
Final backfat depth (mm)	17.20	17.26	1.53	0.987
Backfat depth change (mm)	3.30	3.49	1.50	0.856
Born alive piglets (n)	11.16	10.20	1.42	0.655
Dead piglets (n)[3]	1.50	1.20	0.58	0.437
Pre-weaning mortality (%)	13.44	11.76	3.47	0.563
Weaned piglets (n)	9.66	9.00	0.28	0.284
Piglets BW at birth (kg)	1.60	1.80	0.07	0.145
Piglets BW at weaning (kg)	5.82	6.35	0.41	0.439
ADG of piglets (g/d)	150.15	173.03	7.58	0.082

[1]Values are presented as the least squares mean of 6 replicates.

[2]CON, control sow diet based on corn and soybean meal; SDP, CON + 1% spray-dried plasma; BW, body weight; ADFI, average daily feed intake; ADG, average daily gain; SEM, standard error of mean.

[3]Stillborn and piglets died before weaning.

Table 3. **Immune responses of lactating sows fed diets supplemented with spray-dried plasma**[1]

Item[2]	**Dietary treatments**		**SEM**	***p*-value**
	CON	**SDP**		
Day 1 of lactation				
WBC (x10^3/μL)	11.93	9.55	1.77	0.893
TNF-α (pg/mL)	312.30	289.80	34.19	0.809
TGF-β1 (pg/mL)	357.66	238.16	59.23	0.745
CRP (ng/mL)	241.43	268.87	44.60	0.846
Cortisol (ng/mL)	0.51	0.49	0.06	0.345
Day 3 of lactation				
WBC (x10^3/μL)	15.33	14.08	1.58	0.465
TNF-α (pg/mL)	281.96	264.94	5.06	< 0.10
TGF-β1 (pg/mL)	448.07	311.37	63.21	< 0.10
CRP (ng/mL)	139.95	132.90	18.71	0.723
Cortisol (ng/mL)	0.56	0.48	0.03	< 0.10
Day 7 of lactation				
WBC (x10^3/μL)	13.71	13.98	1.64	0.687
TNF-α (pg/mL)	272.15	249.35	10.21	< 0.10
TGF-β1 (pg/mL)	286.20	257.32	46.00	0.854
CRP (ng/mL)	136.92	105.27	23.21	0.412
Cortisol (ng/mL)	0.49	0.51	0.06	0.597

[1]Values are presented as the least squares mean of 6 replicates.

[2]CON, control sow diet based on corn and soybean meal; SDP, CON + 1% spray-dried plasma; WBC, white blood cells; TNF-α, tumor necrosis factor-α; TGF-β1, transforming growth factor-β1; CRP, C-reactive protein; SEM, standard error of mean.

Table 4. **Immune responses of nursing piglets from sows fed diets supplemented with spray-dried plasma**[1]

Item[2]	Dietary treatments		SEM	*p*-value
	CON	SDP		
Day 3 of lactation				
WBC (x10^3/μL)	10.15	8.54	0.92	0.687
TNF-α (pg/mL)	270.39	262.67	40.86	0.741
TGF-β1 (pg/mL)	788.76	607.40	140.86	0.874
CRP (ng/mL)	72.14	78.15	16.87	0.812
Cortisol (ng/mL)	0.83	0.45	0.23	0.345
Day 7 of lactation				
WBC (x10^3/μL)	13.15	12.98	1.30	0.465
TNF-α (pg/mL)	423.57	349.87	38.99	< 0.10
TGF-β1 (pg/mL)	980.41	853.49	58.99	< 0.10
CRP (ng/mL)	119.42	115.18	26.95	0.586
Cortisol (ng/mL)	1.05	0.62	0.15	< 0.10

[1]Values are presented as the least squares mean of 6 replicates.

[2]CON, control sow diet based on corn and soybean meal; SDP, CON + 1% spray-dried plasma; WBC, white blood cells; TNF-α, tumor necrosis factor-α; TGF-β1, transforming growth factor-β1; CRP, C-reactive protein; SEM, standard error of mean.

Table 5. Serum immunoglobulins of nursing piglets from sows fed diets supplemented with spray-dried plasma[1]

Item[2]	Dietary treatments		SEM	*p*-value
	CON	SDP		
Day 3 of lactation				
IgG (ng/mL)	242.13	251.47	9.95	0.818
IgM (ng/mL)	89.69	106.97	10.16	0.235
IgA (ng/mL)	30.22	25.71	5.38	0.844
Day 7 of lactation				
IgG (ng/mL)	219.28	221.83	13.62	0.795
IgM (ng/mL)	58.46	69.95	7.79	0.612
IgA (ng/mL)	22.65	33.69	4.40	0.692

[1]Values are presented as the least squares mean of 6 replicates.

[2]CON, control sow diet based on corn and soybean meal; SDP, CON + 1% spray-dried plasma; IgG, immunoglobulin G; IgM, immunoglobulin M; IgA, immunoglobulin A; SEM, standard error of mean.

Table 6. Growth performance of weaned pigs from sows fed diets supplemented with spray-dried plasma[1]

	Dietary treatments			
Item[2]	**CON**	**SDP**	**SEM**	***p*-value**
Number of weaned pigs/pen (n)	9.66	9.00		
Initial BW (kg)	5.82	6.35	0.41	0.439
Final BW[3] (kg)	23.23	26.11	0.96	0.102
Feed intake (kg)	25.50	26.85	0.53	0.348
ADG (g/d)	414.52	470.47	18.21	< 0.10
ADFI (g/d)	607.17	639.24	0.14	0.345
G:F ratio (g/g)	0.682	0.735	0.03	0.773

[1]Values are presented as the least squares mean of 6 replicates.

[2]CON, control sow diet based on corn and soybean meal; SDP, CON + 1% spray-dried plasma; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F ratio, gain to feed ratio; SEM, standard error of mean.

[3]The 3-phase feeding program with basal nursery diet was used to feed weaned pigs: week 1 as phase 1; week 2 as phase 2; and week 3 to 6 as phase 3. Pigs had free access to water and diets throughout the experiment.

Table 7. Immune responses of weaned pigs from sows fed diets supplemented with spray-dried plasma[1]

Item[2]	Dietary treatments		SEM	*p*-value
	CON	SDP		
Day 1 postweaning				
WBC (x10^3/μL)	12.91	13.42	1.46	0.412
TNF-α (pg/mL)	320.24	358.90	57.21	0.847
TGF-β1 (pg/mL)	1121.44	1077.46	117.21	0.765
CRP (ng/mL)	87.53	76.54	14.94	0.546
Cortisol (ng/mL)	1.34	1.18	0.10	0.387
Day 3 postweaning				
WBC (x10^3/μL)	16.09	13.44	2.20	0.341
TNF-α (pg/mL)	449.80	344.11	38.32	< 0.10
TGF-β1 (pg/mL)	1071.75	1024.15	158.32	0.687
CRP (ng/mL)	126.60	103.58	17.30	0.541
Cortisol (ng/mL)	1.88	1.40	0.16	< 0.05
Day 7 postweaning				
WBC (x10^3/μL)	16.95	16.15	1.25	0.741
TNF-α (pg/mL)	388.84	370.12	41.89	0.601
TGF-β1 (pg/mL)	836.48	718.33	41.89	< 0.05
CRP (ng/mL)	112.28	78.41	15.50	< 0.10
Cortisol (ng/mL)	1.74	1.56	0.11	0.230

[1)]Values are presented as the least squares mean of 6 replicates.

[2]CON, control sow diet based on corn and soybean meal; SDP, CON + 1% spray-dried plasma; WBC, white blood cells; TNF-α, tumor necrosis factor-α; TGF-β1, transforming growth factor-β1; CRP, C-reactive protein; SEM, standard error of mean.