

## **Effect of dietary supplementation of *Bacillus Subtilis* (C-3102) as a direct feed microbial on sow and nursery pig performance**

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**Abstract:** Three experiments were conducted to determine the effect of a direct feed microbial (DFM) on sow, litter and nursery pig performance. In Expt 1. Fifty-two cross-bred (Yorkshire X Landrace) sows were randomly divided by weight and parity into two dietary treatment groups. The diets were a control corn-soybean meal basal diet or the basal diet supplemented with 0.1% Calsporin<sup>TM</sup> (*Bacillus Subtilis* (C-3102)). These diets were fed to pregnant sows on d 80 of gestation. The sows were fed the same dietary treatments in lactation with supplementation of Calsporin reduced from 0.1% to 0.01% during the 21 d lactation. Fecal samples were collected changes in manure bacteria flora at the end of gestation and at weaning. The results indicated that Calsporin had no effect ( $P>0.05$ ) on sow body weight and backfat changes during gestation and lactation. Sow average feed intake, litter size, birth wt and weaning wt were not influenced by dietary treatments. However, the average daily gain (ADG) of piglets from sows fed Calsporin diet during gestation and lactation was 214.0g, which was significantly greater ( $P<0.05$ ) than 200.4g of the piglets from sows fed the control diet. The number of *Clostridium Perfringens* in the feces of piglets from Calsporin fed sows was less ( $P<0.05$ ) than in the feces of the piglets from the control fed sows (6.13 log vs. 7.13 log, CFU/g feces), The number of *Bifidobacterium* in the feces of piglets from Calsporin fed sows was higher ( $P<.05$ ) than those of piglets from the control fed sows (8.56 log vs. 7.27 log, CFU/g feces). The number of total anaerobic bacteria in the feces of piglets from the sows fed Calsporin diet was less ( $P<0.05$ ) than in the feces of piglets from the sows fed control diet (9.54 log vs. 10.22 log, CFU/g feces). In Experiments 2 and 3, feeding trials were conducted with one hundred and sixty pigs (Expt 2) and one hundred and forty four pigs (Expt 3) in a 2 (lactational feed) X 2 nursery feed factorial design. Weaned pigs from sows fed control diet or the basal + Calsporin were split into two groups, a control (basal) diet or basal diet supplemented with 0.01% Calsporin. In Expt.2, there was no effect of dietary treatment on any of the performance parameters measured. In Expt. 3, pigs fed diets supplemented with Calsporin grew 12.4% and 13.4% faster ( $P<0.02$ ) than the

control fed group. Feeding Calsporin during gestation and lactation had no effect on piglet growth rate during the nursery period. Overall, Calsporin fed pigs grew faster than the control fed pigs. In conclusion, the addition of Calsporin in the diets of late gestation and lactation sow diets increased litter weaning weights. In addition supplementation of Calsporin in nursery diets improved nursery pig performance.

**Key words:** Direct Fed Microbials, Sow and litter performance, Microflora

## **Introduction:**

Intensive rearing conditions for livestock have contributed to the delay or a disturbance in the development of normal intestinal micro flora. Supplementation of “beneficial organisms” to animal feed started in the 1920s and the name “probiotics” was introduced by Parker (1974) when the production of bacterial feed supplements began on a commercial scale (Fuller, 1992a). Numerous studies have reported the benefits of including probiotics or direct fed microbials (DFM) to the diets of young animals to improve their health and nutrition (Whitt and Savage, 1987, Lessard and Brisson, 1987; Chesson, 1994; Stavric and Kornegay, 1995). Manipulation of the gastrointestinal microflora has been suggested as a method to improve the health of young pigs. The interaction between the indigenous bacteria with exogenous DFM in young pigs can either prevent or suppress pathogenic bacteria from colonizing the intestinal tract. Abe, et. al., (1955) and Kyriakis, et. al., (1999) reported that piglets given feed with supplemented with probiotic (*Bacillus licheniformis*) exhibited a reduced incidence and severity of diarrhea and mortality, and increased weight gain and feed conversion ratio. The normal microflora colonized the digestive tract in livestock species at five to six days after birth (Vanbelle, et. al., 1990). Feed supplementation with beneficial bacteria increases their numbers in the digestive tract. . Altherton and Robbins (1987) defined probiotic as any product which can help the normal flora to maintain their domination over pathogenic organisms. The objective of this study was to determine the effects of dietary supplementation of a probiotic, Calsporin<sup>TM</sup> (*Bacillus subtilis* C-3102) on the performance of sow and litter, early weaned pigs and microflora changes in feces of sows and piglets.

## **Materials and Methods**

The University of Minnesota Institutional Animal Care and Use Committee approved the materials and methods used in these experiments.

### **Experiment 1:**

#### **Gestation and Lactation Sows:**

Fifty three multiparous cross-bred sows (Yorkshire x Landrace) were used in the experiment. Corn-soybean meal based gestation and lactation diets (Table 1 ) were formulated to meet or exceed suggested requirements for all nutrients (NRC, 1988). Sows were fed 1.6 kg (as-fed basis) of the gestation diet twice daily during the final 30 d of gestation. The lactation diet was provided from d 100 of gestation and throughout the 21 d lactation period. The dietary treatments were the standard corn-soybean meal diet (basal diet or Control) or the basal diet supplemented with 0.1% Calsporin (Calsporin). Twenty-six sows and 27 sows were fed the control and Calsporin diets respectively from d 80 – d 110 of gestation. During gestation, sows were housed in groups of six per pen. To avoid fecal contamination, adjacent pens were left vacant. Sows were weighed and ultrasonic backfat measurement taken on d 80 of gestation.

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On d 110 of gestation, the sows were placed in individual farrowing crates (1.6 x 2.4 –m) in an environmentally controlled room. After parturition, farrowing, sows were fed their lactation treatment diets with 0.01% Calsporin supplemented diet or the control. Lactation feed was restricted within the first 72 h after farrowing. The diets were fed initially at 3.0 kg, but 1.5 kg of feed was cumulatively added each subsequent day until 3 d postpartum, when they were provided their diet for ad libitum consumption to weaning (d 21 postpartum). Water was available at all times. Sow and litter weights were determined within 12 h after parturition and at weaning. Sow backfat thickness (Renco Lean Meater, Minneapolis, MN) at the last rib were determined within 12 hr after parturition and at weaning (d 21 postpartum). Litter size at birth and at weaning, number of mummified, stillborn pigs and pre-weaning mortality were recorded. Piglets did not have access to creep feed. . The weaning to mating interval (day) was recorded. ..

## Experiment 2 and 3

In Exp. 2, 168 pigs (PIC : C22 x Duroc; average  $20 \pm 2$  d of age and  $7.7 \pm 0.21$  kg) were blocked based on sow lactation dietary treatment, initial weight, sex and ancestry and randomly allotted to two dietary treatments for a 28-d study. Pigs were housed in an environmentally regulated building with slatted flooring over a pit. Each pen (1.2 x 1.2m) had a stainless steel nipple drinker and a stainless steel self-feeder. There were seven pigs per pen, utilizing seven pens per dietary treatment. The two dietary treatments were created by supplementing the phase 1 and 2 basal diets with 0.01 Calsporin. Pigs had ad libitum access to water and feed. Body weights and pen consumption were measured at the end of each phase to evaluate ADG, ADFI and G:F. All diets were fed in meal form and all nutrients requirements met or exceeded NRC (1998) requirements estimates for the nursery pig (Table 1).

In Exp 3, 144 pigs (PIC: C22 x Duroc; average  $20 \pm 2$  d of age and  $7.6 \pm 0.22$  kg) were blocked based on sow lactation dietary treatment, initial weight, sex and ancestry and randomly allotted to two dietary treatments for a 28-d study. There were six pigs per pen, utilizing six pens per dietary treatment. The dietary treatments and animal care were the same as for Exp 2.

**Microbial identification:** Fecal grab samples were collected twice (0800 and 1530) on d 18 post-partum from 10 sows and 5 pigs per litter per dietary treatment. The fecal samples were stored in sterile bags at 0 °C. Fecal samples were diluted with PBS at  $10^5$  to  $10^7$  times and cultured with specific medium in trays (Qbiogene, Inc. Carlsbad, CA. Enterobacteriaceae, Clostridium Perfringens, Streptococcus, Lactobacillus, Bifidobacterium, Total bacteria (anaerobe), Calsporin. The number of Clostridium Perfringens and Bifidobacterium in the feces was counted and CFU/g was calculated. ....

### Statistical Analysis

Data were analyzed as a randomized complete block design using the GLM procedures of SAS (SAS Inst. Inc. Cary, N.C.)

## **Results**

### **The performance of sows and pigs**

The performance of sows during gestation and lactation were not influenced by dietary treatments (Table 3). The average body weight gain of sows during gestation (d 80 to d 110 of gestation), were  $17.4 \pm 10.2$  to  $19.0 \pm 10.9$  kg for the control and Calsporin groups respectively. There was no significant difference ( $P > 0.05$ ) in backfat measurements between treatments during gestation and lactation (Table 3).

The sow lactation body weight loss were not affected ( $P > 0.05$ ) by dietary treatments (6.4 kg for sows fed control diet and 7.3 kg for the sows fed Calsporin diet, respectively). The daily feed intake was not influenced by dietary treatments ( $5.6 \pm 1.2$  kg/d and  $5.2 \pm 1.0$  kg/d for the control and Calsporin fed sows, respectively). The weaning to mating intervals was  $7.1 \pm 3.5$  and  $7.4 \pm 3.7$  days for control and Calsporin treatments ( $P > .05$ ) respectively.

The average daily gain of piglets from the sows fed Calsporin diet was greater ( $P < 0.05$ ) than that from the sows fed control diet (214.0 g vs. 200.4 g). The percentages of mummified (9.01% vs. 5.88%) and stillborn (8.11% vs. 6.81%) pigs from the sows fed Calsporin diet were less than those from the sows fed control diet (Table 3). However, The survival rate of piglets from Control and Calsporin sows at weaning (88.3% vs. 87.4%) was not different ( $P > .05$ ). Dietary probiotic treatment had no effect on piglet birth weight.

### **Fecal microflora changes in sows and pigs**

The amount of *Clostridium Perfringens* in the first fecal collection of sows fed control diet was higher ( $P < .05$ ) than sows fed Calsporin diet (7.13 log vs. 6.13 log CFU/g feces), and the feces from sows fed Calsporin had more ( $P < .05$ ) *Bifidobacterium* than the sows fed control diet (8.56 log vs. 7.27 log CFU/g feces). The number of *Enterobacteriaceae*, *Streptococcus* and *Lactobacillus* in the feces of sows was not different ( $P > .05$ ) between the dietary treatments (Table 4). The total anaerobic bacteria in the feces of pigs from Calsporin fed sows was less ( $P < .05$ ) than pigs from control sows

(9.54 log vs. 10.22 log CFU/g feces), but other bacteria were not different between the two derivative pigs. **Calsporin was higher** ( $P < .001$ ) in the Calsporin treated feces for both sows and pigs.

For the second fecal collection, the amount of examined bacteria in the feces of sows and pigs was not influenced by dietary treatments ( $P > .05$ ). However, the total anaerobic bacteria in the feces of pigs from Calsporin fed sows was less ( $P < .05$ ) than pigs from control sows (9.54 log vs. 10.13 log CFU/g feces), and Calsporin was higher in the Calsporin treated feces for both sows ( $P < .01$ ) and pigs ( $P < .05$ ).

## **Discussion**

Probiotics have been used to improve pig growth performance and health through an effect on the gut or gut flora. Results of the current study showed that sows fed probiotic diet had less mummified and stillborn pigs, which implies improved sow health status during pregnancy. Probiotics enhanced the good indigenous microflora that was helpful for both digestive tract and the health of the whole body.

Pig performance was improved by Calsporin supplementation in the sow diet during the latter part of gestation and lactation. Body weight gain of piglets was significantly greater than those from the control sows, which indicated that the neonate pigs benefited from the sows fed Calsporin diet during gestation and lactation. The piglets might have consumed the sows feed or feces with the.

Probiotic did not influence the reproductive performance of the sows.. This need could be met by an absolute increase in amount of nutrients absorbed, by nutrient sparing or by the repartitioning of nutrients already available to the host (Chesson, 1994). Reports of the influence of probiotic feeding on nutrient digestibility and N retention are not consistent (Stavric and Kornegay, 1995).

## **Conclusion**

The experiments did not show the effects of Calsporin on the performance of sows during late gestation and lactation. However, Calsporin increased the number of

Bifidobacterium in the feces of sows and decreased the number of total anaerobic bacteria in the feces of piglets, which may be of benefit to animals and may be the reason of the growth improvement for pigs during suckling period.

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Table 1. Composition of basal diets for sows (Exp. 1)

Ingredients (%)	Gestation		Lactation	
	Control	Calsporin	Control	Calsporin
Corn	78.3	78.2	61.5	61.5
SBM	16.2	16.2	32.0	32.0
Tallow	1.0	1.0	1.0	1.0
Di-calcium phosphate	1.5	1.5	2.0	2.0
Limestone	0.9	0.9	0.9	0.9
Salt	0.5	0.5	0.5	0.5
Vitamin – mineral premix	0.25	0.25	0.25	0.25
Lysine	---	---	0.01	0.01
Calsporin <sup>TM</sup>	---	0.1	---	0.01
Calculated nutrient analysis				
ME (KJ/kg)	12.5	12.5	13.2	13.2
CP (%)	13.5	13.4	16.8	16.75
Ca (%)	0.8	0.8	0.85	0.85
P (%)	0.75	0.75	0.78	0.78
Lysine (%)	0.55	0.55	0.95	0.95

<sup>1</sup> Premix supplied the following per kg of diet: Zn, 120 mg; Mn, 12 mg; Fe, 150 mg; Cu, 12 mg; Se, 1 mg; vitamin A, 5,000 IU; Vitamin D<sub>3</sub>, 500 IU; vitamin E, 22 IU; riboflavin, 12 IU; nicacin 45mg; calcium pantothenate, 24 mg; choline chloride, 840 mg; vitamin B<sub>12</sub>, 30 µg; biotin, 200 µg.

Table 2. Composition of basal diets for piglets (Exp. 2 & 3)

Ingredients (%)	Phase 1		Phase 2	
	Control	Calsporin	Control	Calsporin
Corn	48.19	48.18	51.35	51.35
SBM	15.00	15.00	25.00	25.00
Fish meal	12.00	12.00	4.00	4.00
Whey powder	10.00	10.00	6.00	6.00
Oats	10.00	10.00	8.00	8.00
Tallow	2.85	2.85	3.00	3.00
Premix <sup>1</sup>	0.25	0.25	0.25	0.25
Di-calcium phosphate	0.50	0.50	0.50	0.50
Limestone	0.40	0.40	1.00	1.00
Salt	0.50	0.50	0.50	0.50
Lysine	0.20	0.20	0.20	0.20
Threonine	0.10	0.10	0.10	0.10
Tryptophan	0.01	0.01	0.01	0.01
Calsporin <sup>TM</sup>	-	0.01	-	0.01
Calculated nutrient analyses				
ME (KJ/kg)	13.40	13.40	13.81	13.81
CP (%)	20.80	21.00	19.50	19.70
Ca (%)	1.25	1.25	0.91	0.91
P (%)	0.67	0.67	0.62	0.62
Lysine (%)	1.44	1.44	1.17	1.17

<sup>1</sup>Premix supplied the following per kg of diet: Zn, 120 mg; Mn, 12 mg; Fe, 150 mg; Cu, 12 mg; Se, 1 mg; vitamin A, 5,000 IU; Vitamin D<sub>3</sub>, 500 IU; vitamin E, 22 IU; riboflavin, 12 IU; nicacin 45mg; calcium pantothenate, 24 mg; choline chloride, 840 mg; vitamin B<sub>12</sub>, 30 µg; biotin, 200 µg.

Table 3. Effect of Calsporin™ on sow performance (Exp. 1)

	<b>Control</b>	<b>Calsporin</b>	<b>SEM</b>	<b>Sign.</b>
Number of sows.	26	27		
<i>Sow weights (kg)</i>				
D 90 gestation	237.50	229.70	6.10	0.65
D 109 gestation	243.50	237.90	6.30	0.48
D 0 Lactation	217.40	206.90	5.90	0.30
At weaning	209.00	201.70	6.10	0.31
<i>Sow backfat depth (mm)</i>				
D 90 gestation	12.70	11.80	0.60	0.53
D 109 gestation	12.40	11.50	0.60	0.52
D 0 Lactation	12.30	11.40	0.60	0.54
At weaning	11.60	10.97	0.60	0.71
Average sow feed intake (kg/day)	5.55	5.22	0.18	0.31
Wean to mating interval (day)	7.05	7.36	0.65	0.78

Note: Figures with different superscript letters were significant differences (P<0.05).

Table 4. The influence of dietary Calsporin<sup>TM</sup> in sow diets on piglet performance (Exp. 1)

	<b>Control</b>	<b>Calsporin</b>	<b>SEM</b>	<b>Sign.</b>
Number of Sows	26	27		
Lactation length (d)	20.16	19.62	0.43	0.53
Litter size at birth	11.44	11.14	0.48	0.95
Litter size at weaning	10.10	9.73	0.22	0.26
Initial BW of piglets (g)	1495	1481	16.41	0.16
BW of piglets at weaning (g)	5404	5615	66.16	0.50
Average daily gain of piglets (g)	200.4 <sup>b</sup>	214.0 <sup>a</sup>	3.04	0.02
Mortality and weak pigs (%)	6.31	7.74		
Percentage of mummy pigs (%)	9.01	5.88		
Percentage of stillborn (%)	8.11	6.81		

Note: Figures with different superscript letters were significant differences (P<0.05).

**Table 4.** The effect of Calsporin™ on the performance of weaned piglets (Exp. 2)

Sows Dietary Treatment	Sows fed Control diet				Sows fed Calsporin diet			
Piglets Dietary Treatment	Contr.	Calsp.	SEM	Sig.	Contr.	Calsp.	SEM	Sig.
Number of pigs	42	42			42	42		
<b>Phase 1 (0-14d)</b>								
Initial Body Wt. (kg)	6.02	6.02	0.19	---	6.45	6.38	0.13	---
14d Body Wt. (kg)	7.57	7.87	0.23	0.33	7.81	8.12	0.19	0.31
ADG (g/d)	111.0	132.2	11.7	0.77	97.4	124.3	10.2	0.61
Feed Intake (g/d)	196.5	215.5	12.2	0.83	175.5	208.8	12.3	0.53
Gain/Feed	0.56	0.61	0.08	0.35	0.56	0.59	0.08	0.24
<b>Phase 2, 14-28d</b>								
28d Body Wt. (kg)	13.02	13.63	0.42	0.64	13.03	13.41	0.37	0.53
ADG (g/d)	389.1	411.0	17.9	0.35	372.8	378.0	17.3	0.99
Feed Intake (g/d)	610.9	641.1	12.8	0.69	577.8	604.8	13.0	0.23
Gain/Feed	0.63	0.64	0.02	0.89	0.65	0.63	0.02	0.32
<b>Overall, 0-28d</b>								
ADG (g/d)	250.0	271.6	14.5	0.09	235.1	251.2	13.3	0.52
Feed Intake (g/d)	417.5	431.8	19.5	0.18	392.6	412.0	19.9	0.16
Gain/Feed	0.60	0.63	0.05	0.23	0.60	0.61	0.05	0.31

Table 5. The effect of Calsporin on the productivity of weaned piglets (Exp. 3)

Sows Dietary Treatment	Sows fed Control diet				Sows fed Calsporin diet			
Piglets Dietary Treatment	Contr.	Calsp.	SEM	P <sub>value</sub> .	Contr.	Calsp.	SEM	P <sub>value</sub> ..
Number of pigs	36	36			36	36		
<b>Phase 1 (0-14d)</b>								
Initial Body Wt. (kg)	5.77	5.71	0.18	---	6.43	6.44	0.17	--
14d Body Wt. (kg)	7.37	7.60	0.26	0.33	7.59	7.96	0.22	0.25
ADG (g/d)	127.2	140.2	10.0	0.13	103.7	117.8	8.4	0.28
Feed Intake (g/d)	198.5	213.4	8.8	0.56	179.3	202.5	6.9	0.19
Gain/Feed	0.62	0.63	0.27	0.89	0.59	0.58	0.04	0.54
<b>Phase 2 (14-28d)</b>								
28d Body Wt. (kg)	12.64	13.57	0.49	0.36	12.74	13.96	0.39	0.31
ADG (g/d)	387.7 <sup>b</sup>	435.9 <sup>a</sup>	21.5	0.04	377.8 <sup>b</sup>	428.6 <sup>a</sup>	17.5	0.02
Feed Intake (g/d)	600.9	641.6	14.5	0.43	600.1	646.0	12.7	0.24
Gain/Feed	0.64 <sup>a</sup>	0.68 <sup>b</sup>	0.02	0.05	0.63 <sup>a</sup>	0.67 <sup>b</sup>	0.02	0.05
<b>Overall (0-28d)</b>								
ADG (g/d)	257.4 <sup>b</sup>	288.1 <sup>a</sup>	15.2	0.04	240.7 <sup>b</sup>	273.2 <sup>a</sup>	17.1	0.04
Feed Intake (g/d)	399.7	427.5	18.9	0.42	389.7	424.2	20.2	0.13
Gain/Feed	0.63	0.65	0.03	0.38	0.61	0.63	0.04	0.90

a-b Means with different superscripts are significantly different (P<0.05)

Table 5. Microflora changes in feces of sows and pigs in 1<sup>st</sup> phase (log CFU/g  $\pm$ SD)

	Sows			Pigs		
	Control	Calsporin	Sig.	Control	Calsporin	Sig.
No. of animals	10	10		5	5	
Enterobacteriaceae	6.76 $\pm$ 0.4	6.77 $\pm$ 0.78	NS	8.05 $\pm$ 0.8	7.55 $\pm$ 0.4	NS
Clostridium Perfringens	7.13 $\pm$ 0.4	6.13 $\pm$ 1.62	*	8.26 $\pm$ 0.3	7.75 $\pm$ 1.4	NS
Streptococcus	7.32 $\pm$ 0.5	7.54 $\pm$ 0.69	NS	8.09 $\pm$ 1.4	7.61 $\pm$ 0.4	NS
Lactobacillus, A	8.78 $\pm$ 0.1	8.77 $\pm$ 0.33	NS	8.57 $\pm$ 1.4	8.30 $\pm$ 0.5	NS
Bifidobacterium, B	7.27 $\pm$ 0.4	8.56 $\pm$ 0.84	*	7.82 $\pm$ 0.5	8.13 $\pm$ 0.9	NS
Total bacteria (anaerobe), C	9.26 $\pm$ 0.2	9.24 $\pm$ 0.23	NS	10.2 $\pm$ 0.2	9.54 $\pm$ 0.1	*
A/C	41.0 $\pm$ 24.5	39.8 $\pm$ 20.7	NS	7.71 $\pm$ 10.	9.14 $\pm$ 8.2	NS
B/C	1.40 $\pm$ 0.8	46.5 $\pm$ 41.8	*	0.87 $\pm$ 1.1	8.21 $\pm$ 6.1	NS
Calsporin	4.62 $\pm$ 0.10	7.17 $\pm$ 0.12	***	2.83 $\pm$ 0.2	5.32 $\pm$ 0.5	**

Note: \*: P<0.05; \*\*: P<0.01; \*\*\*: P<0.0001.

Table 6. Microflora changes in feces of sows and pigs 2<sup>nd</sup> phase (log CFU/g ±SD)

	Sows			Pigs		
	Control	Calsporin	Sig.	Control	Calsporin	Sig.
No. of animals	10	10		5	5	
Enterobacteriaceae	6.91±0.13	6.70±0.53	NS	7.88±0.91	7.55±0.49	NS
Clostridium Perfringens	7.27±0.45	6.50±2.01	NS	8.17±0.28	7.75±1.43	NS
Streptococcus	7.45±0.55	7.74±0.70	NS	7.65±1.29	7.61±0.49	NS
Lactobacillus, A	8.69±0.13	8.79±0.23	NS	8.49±1.67	8.30±1.50	NS
Bifidobacterium, B	7.48±0.58	8.50±1.03	NS	8.23±0.85	8.13±0.91	NS
Total bacteria (anaerobe), C	9.46±0.27	9.29±0.31	NS	10.1±0.47	9.54±0.14	*
A/C	22.0±20.5	36.6±20.4	NS	9.15±10.9	9.14±8.26	NS
B/C	1.62±0.85	44.7±43.8	NS	1.67±0.15	8.21±6.03	NS
Calsporin	4.66±0.03	7.15±0.13	***	2.83±0.28	5.32±0.50	*

Note: \*: P<0.05; \*\*: P<0.01; \*\*\*: P<0.0001.