Kansas Agricultural Experiment Station Research Reports

Volume 3
Issue 7 *Swine Day*

Article 9

2017

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Recommended Citation

Menegat, M. B.; Vier, C. M.; Cemin, H. S.; Shawk, D.; DeRouchey, J. M.; Woodworth, J. C.; Tokach, M. D.; Dritz, S. S.; and Goodband, R. D. (2017) "Effect of Calsporin on Nursing Piglet Growth Performance and Fecal Microflora," *Kansas Agricultural Experiment Station Research Reports*: Vol. 3: Iss. 7. https://doi.org/10.4148/2378-5977.7462

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Funding Source

Appreciation is expressed to Calpis Co., Ltd. (Tokyo, Japan), and Quality Technology International, Inc. (Elgin, IL) for microbial analysis and financial support.

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Effect of Calsporin on Nursing Piglet Growth Performance and Fecal Microflora[1]

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Summary

A total of 26 lactating sows (DNA 241, DNA Genetics, Columbus, NE) and litters were used in a discovery study to evaluate the effects of Calsporin, a direct-fed microbial containing *Bacillus subtilis* C-3102, on fecal microflora of nursing pigs. The treatments consisted of providing a daily oral dose of Calsporin or a placebo control to piglets during the nursing phase. Sows were randomly assigned to treatments based on farrowing date, parity, and initial BW. The treatments were applied individually to piglets once a day from d 2 after farrowing and equalization until weaning on d 19. Sow BW, sow ADFI, piglet BW, piglet weight gain, litter size, and mortality were recorded on a weekly basis until weaning. Fecal scoring was conducted to categorize the consistency of the feces using a numerical scale from 1 to 5. Also, fecal samples were collected directly from the rectum of the piglets and pooled by litter for microbial analysis. Fecal scoring and microbial analysis were performed on d 2 after birth, and after 1 or 2 weeks of treatment. As expected, and not a primary objective of this study, there was no evidence for differences among treatments on sow and litter performance ($P > 0.085$). There was no evidence for differences on fecal score at the beginning of the trial and after 1 or 2 weeks of Calsporin supplementation ($P > 0.358$). Microbial analysis revealed an increase in levels of total *Bacillus* sp. ($P < 0.001$) and a decrease in total aerobes ($P < 0.026$) in litters treated with Calsporin. There was no evidence for differences in number of *Lactobacillus* sp., *Enterococcus* sp., *Clostridium perfringens*, Enterobacteriaceae, and total anaerobes between control- and Calsporin-treated litters ($P > 0.05$). In conclusion, once per day supplementation of Calsporin to nursing pigs resulted in slight changes in fecal microflora, but there was no influence on nursing pig fecal consistency.

Introduction

Feeding strategies to improve performance and preserve health in pigs while minimizing the use of antibiotics is of great interest for the swine industry. Probiotics are non-pathogenic live microorganisms that, once ingested in adequate amounts, can improve the intestinal microbial balance and benefit the animal.[3] The beneficial effects of probi-

[1] Appreciation is expressed to Calpis Co., Ltd. (Tokyo, Japan), and Quality Technology International, Inc. (Elgin, IL) for microbial analysis and financial support.

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[3] Fuller, R. 1989. Probiotics in man and animals. J. Appl. Bacteriol. 66:365-378.

otics on intestinal health include the development of a healthy microbiota, prevention of colonization by enteric pathogens, increase in digestive capacity, reduced intestinal pH, and improvement in gut immunity, maturation, and integrity.[4] The strains of bacteria that are typically used as probiotics for livestock animals include *Lactobacillus* sp., *Bifidobacterium* sp., *Enterococcus* sp., and *Bacillus* sp.[5] *Bacillus* sp. are Gram-positive spore-forming bacteria that are used as probiotics either as single strain or multi-strain preparations. Spores are considered stable during feed manufacturing and storage, and after ingested can germinate but not proliferate in the intestine.

Calsporin (Calpis Co. Ltd., Tokyo, Japan) is a direct-fed microbial product based on viable spores of a non-genetically modified strain, *Bacillus subtilis* C-3102. In swine, *Bacillus subtilis* C-3102 has been fed as a growth promoter in nursery pigs[6] and is thought to work by increasing the populations of the beneficial intestinal microflora, particularly the *Lactobacillus* sp.[7] However, this product has not been directly administered to piglets while they are nursing to determine if improved nursing performance or fecal microflora can be observed. Therefore, the objective of the present study was to evaluate the effect of a daily oral dose of Calsporin on fecal microflora of nursing pigs.

Procedures

The Kansas State University Institutional Care and Use Committee approved the protocol used in this experiment. The experiment was conducted at the Kansas State Swine Teaching and Research Center in Manhattan, KS. A total of 26 lactating sows (DNA 241, DNA Genetics, Columbus, NE) and litters were used in the study. The average parity was 2.5. At d 110 of gestation, sows were weighed and individually housed in an environmentally-controlled and mechanically-ventilated farrowing house.

Sows and litters were randomly assigned to treatments based on farrowing date and parity. Piglets were cross-fostered within 24 h after birth to equalize litter size. Treatments began on d 2 after birth and continued until weaning at approximately d 19 of age. Therefore, d 2 and 19 of age corresponded to d 0 and 17 of the trial, respectively. The treatments consisted of either a placebo control (n = 14 litters) or supplementation of Calsporin (Calpis Co. Ltd., Tokyo, Japan) in the control suspension (n = 12 litters). The treatments were applied orally to individual piglets once daily at approximately 0700 h. Daily Calsporin dosage was 45.0×10^6 CFU/mL, 77.5×10^6 CFU/mL and 108.3×10^6 CFU/mL for d 0 to 7, d 7 to 14, and d 14 to weaning, respectively, administered via 1 mL liquid suspension. The liquid suspension was based on a carrier (Headstart, Animal Science Products, Inc., Nacogdoches, TX, US) alone or with Calsporin. The preparation of treatments consisted of dissolving the products in warm water while continuously mixing the solution with a magnetic stirrer. The solution was prepared

[4] De Lange, C.F.M., Pluske, J., Gong, J., and Nyachoti, C.M. 2010. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. Livest. Sci. 134:124-134.
[5] Ohashi, Y., and Ushida, K. 2009. Health-beneficial effects of probiotics: Its mode of action. Anim. Sci. J. 80:361-371.
[6] Marubashi, T., Gracia, M.I., Vilà, B., Bontempo, V., Kritas, S.K., and Piskoríková, M. 2012. The efficacy of the probiotic feed additive Calsporin® (*Bacillus subtilis* C-3102) in weaned piglets: Combined analysis of four different studies. J. Appl. Anim. Nut. 1:1-5.
[7] Maruta, K., Miyazaki, H., Tadano, Y., Masuda, S., Suzuki, A, Takahashi, H., and Takahashi, M. 1996. Effects of *Bacillus subtilis* C-3102 intake on fecal flora of sows and on diarrhea and mortality rate of their piglets. Anim. Sci. Technol. 67(5):403-409.

immediately before use and given in one oral dose of 1 mL per piglet. Both control and treatment suspensions were analyzed for quantification of *Bacillus subtilis* C-3102.

Sows were allowed ad libitum feed intake during lactation with daily feed delivered and recorded by an electronic feeding system (Gestal Solo Feeders Jyga Technologies, Quebec City, Quebec, Canada). Sow BW was measured on d 2 post-farrowing and weekly until weaning. Piglets were individually weighed on d 2 after birth and on a weekly basis until weaning. Fecal scoring was conducted on d 2 after birth, and after 1 and 2 weeks of treatment to categorize the consistency of the feces per litter using a numerical scale from 1 to 5: 1, hard feces like pellet; 2, firm formed stool; 3, soft moist stool that retains shape; 4, soft unformed stool; and 5, watery liquid stool.

Microbial analysis was performed on fecal samples pooled by litter. Fecal samples were collected directly from the rectum of the piglets using mini cotton tip swabs on d 2 after birth, and after 1 and 2 weeks of treatment. Microbial analysis of fecal samples was performed by culture method and quantification ($\log_{10}$ CFU/g) of Calsporin (*Bacillus subtilis* C-3102), *Lactobacillus* sp., *Clostridium perfringens*, *Salmonella* spp., *Enterococcus* sp., Enterobacteriaceae, total *Bacillus* sp., total aerobes, and total anaerobes. Limit of detection for Calsporin, *Salmonella* spp. and total *Bacillus* sp. was 2×10^2.

Data were analyzed using a linear mixed model. Treatment was included as fixed effect and block was included as random effect. Litter was the experimental unit. Sow BW, sow ADFI, piglet BW, piglet weight gain, and litter size were fitted assuming a normal distribution of the response variables. Mortality was fitted assuming a binomial distribution. Fecal score and microbial analysis were analyzed as repeated measures. Statistical models were fitted using the GLIMMIX procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC). Results were considered significant at $P \leq 0.05$.

Results and Discussion

Quantification of *Bacillus subtilis* C-3102 in the oral products that were provided daily to the piglets revealed undetectable levels in the control, and 7.95×10^8 CFU/g, 10.40×10^8 CFU/g and 9.84×10^8 CFU/g in the Calsporin treatment for d 0 to 7, d 7 to 14, and d 14 to 17 of the trial, respectively.

While not a primary objective due to limited replication, sow and litter performance was measured. There was no evidence for differences on sow BW ($P > 0.085$; Table 1) or ADFI ($P > 0.342$) during lactation among treatments. For litter performance, no evidence for differences was observed on piglet BW ($P > 0.067$) or weight gain ($P > 0.163$) during lactation and no evidence for differences was observed on litter size ($P > 0.060$) or mortality ($P > 0.334$) throughout the study. Fecal score was reduced from d 0 to 7 and increased from d 7 to 14 in both treatments, suggesting hardening of feces during the first week and softening of feces on the second week of trial, respectively. However, there was no evidence for differences on fecal score between control and Calsporin-treated litters ($P > 0.358$; Table 1).

Microbial analysis revealed a change in the numbers of Calsporin (*Bacillus subtilis* C-3102), total *Bacillus* sp., and total aerobes ($P < 0.05$; Table 2) in litters treated with Calsporin. The detected level of Calsporin was affected by the interaction between treatment and day ($P < 0.001$), where the number of *Bacillus subtilis* C-3102 was increased to a greater extent in litters supplemented with Calsporin compared to controls on d 7 and 14 of treatment. Similarly, an interaction between treatment and day ($P < 0.001$) showed the number of total *Bacillus* sp. was increased in litters supplemented with Calsporin compared to controls on d 7 and 14 of treatment, but not on d 0. Both the levels of Calsporin and total *Bacillus* sp. in the controls increased over time, whereas levels in the Calsporin-treated litters increased from d 0 to 7 and remained constant until d 14 ($P < 0.001$). The number of total aerobes was reduced in piglets treated with Calsporin for 14 d ($P < 0.026$) and decreased from d 0 to 14 in both control and Calsporin-treated piglets ($P < 0.001$).

The number of *Lactobacillus* sp. increased from d 0 to 7 and decreased from d 7 to 14 ($P < 0.001$) in both treatments, whereas the detected levels of *Enterococcus* sp., Enterobacteriaceae, and total anaerobes decreased from d 0 to 14 ($P < 0.001$) in both treatments. There was no evidence for differences in number of *Lactobacillus* sp., *Enterococcus* sp., Enterobacteriaceae, and total anaerobes between control and Calsporin-treated litters ($P > 0.05$). The detected level of *Clostridium perfringens* remained constant during the period of treatment, with no evidence for difference between treatments ($P > 0.331$). The analysis revealed non-detectable levels of *Salmonella* spp. in the feces (limit of detection 2×10^2 CFU/g), except for one sample on d 0 in the control treatment (2.75×10^7 CFU/g).

In conclusion, daily supplementation of Calsporin to nursing pigs resulted in slight changes in fecal microflora by increasing total *Bacillus* sp. and decreasing total aerobes but did not influence fecal consistency.

Table 1. Effects of orally dosing piglets with Calsporin on sow and litter performance[1]

Item	Control	Calsporin	P <
Sow BW, lb			
d 0	489.6 ± 11.7	510.9 ± 12.7	0.181
d 7	482.9 ± 10.1	503.9 ± 10.8	0.135
d 14	478.4 ± 10.5	502.5 ± 11.2	0.099
d 17	476.1 ± 9.7	498.5 ± 10.3	0.085
Sow feed intake, lb			
d 0 to 7	11.3 ± 0.5	12.0 ± 0.5	0.342
d 7 to 14	15.7 ± 0.4	16.0 ± 0.4	0.648
d 14 to 17	17.4 ± 0.6	17.2 ± 0.6	0.772
d 0 to 17	14.3 ± 0.4	14.6 ± 0.4	0.570
Piglet BW, lb			
d 0	3.58 ± 0.09	3.38 ± 0.09	0.067
d 7	6.65 ± 0.17	6.51 ± 0.18	0.489
d 14	10.71 ± 0.29	10.31 ± 0.31	0.283
d 17	12.25 ± 0.35	11.93 ± 0.37	0.484
Piglet weight gain, lb			
d 0 to 7	3.06 ± 0.11	3.17 ± 0.12	0.510
d 7 to 14	4.06 ± 0.15	3.80 ± 0.16	0.163
d 14 to 17	1.52 ± 0.13	1.61 ± 0.14	0.555
Total	8.67 ± 0.30	8.59 ± 0.33	0.849
Litter size, n			
d 0	16.0 ± 0.22	15.7 ± 0.23	0.310
d 7	15.7 ± 0.22	15.1 ± 0.23	0.060
d 14	14.9 ± 0.22	14.8 ± 0.23	0.803
d 17	14.8 ± 0.25	14.7 ± 0.27	0.923
Mortality, %			
d 0 to 7	1.79 ± 0.88	3.72 ± 1.38	0.445
d 7 to 14	5.00 ± 1.47	1.66 ± 0.95	0.334
d 14 to 17	0.96 ± 0.67	0.56 ± 0.56	0.738
Total	7.47 ± 1.88	5.79 ± 1.79	0.512
Fecal score[2]			
d 0	2.08 ± 0.20	2.11 ± 0.22	0.943
d 7	1.66 ± 0.14	1.52 ± 0.15	0.500
d 14	2.08 ± 0.20	2.36 ± 0.22	0.358

[1] A total of 26 sows (DNA 241, DNA genetics) were used in a nursing trial with 14 sows for control and 12 sows for Calsporin treatment. Calsporin (Calpis Co. Ltd., Tokyo, Japan) is a direct-fed microbial based on viable spores of *Bacillus subtilis* C-3102. Pigs were given one daily oral dose of Calsporin from d 0 to 17, for a total of 17 days of treatment. Day 0 corresponded to day 2 after birth and d 17 corresponded to the day of weaning at ~19 days of age. Calsporin dosage was 45×10^6 CFU/mL, 77.5×10^6 CFU/mL and 108.3×10^6 CFU/mL for d 0 to 7, d 7 to 14 and d 14 to wean, respectively. Values are mean ± standard error of the mean.

[2] Consistency of the piglet feces per litter using a numerical scale from 1 to 5: 1, hard feces like pellet; 2, firm formed stool; 3, soft moist stool that retains shape; 4, soft unformed stool; and 5, watery liquid stool.

Table 2. Effects of orally dosing piglets with Calsporin on fecal microflora[1]

	Control			Calsporin			*P* <		
Bacteria	d 0	d 7	d 14	d 0	d 7	d 14	Treatment	Day	Trt × Day
Calsporin[2, 3]	2.02^{xa}	2.36^{ya}	3.20^{za}	2.24^{xb}	5.55^{yb}	5.74^{yb}	<0.0001	<0.0001	<0.0001
SEM	0.06	0.10	0.08	0.06	0.11	0.08			
Detected/sampled	2/14	7/14	14/14	7/12	12/12	12/12			
Total *Bacillus* sp.[3]	2.44^{x}	3.32^{ya}	3.75^{za}	2.67^{x}	5.55^{yb}	5.75^{yb}	<0.0001	<0.0001	<0.0001
SEM	0.13	0.10	0.12	0.13	0.11	0.12			
Detected/sampled	10/14	14/14	14/14	11/12	12/12	12/12			
Lactobacillus sp.	7.84	8.85	8.48	8.04	8.84	8.45	0.620	<0.0001	0.731
SEM	0.16	0.06	0.10	0.19	0.06	0.11			
Detected/sampled	14/14	14/14	14/14	11/11	12/12	12/12			
Clostridium perfringens	8.74	8.79	8.59	8.72	8.84	8.89	0.331	0.672	0.413
SEM	0.02	0.13	0.15	0.02	0.14	0.17			
Detected/sampled	14/14	14/14	14/14	12/12	12/12	12/12			
Enterococcus sp.	8.58	7.59	5.41	8.74	7.25	6.70	0.181	<0.0001	0.103
SEM	0.11	0.19	0.52	0.11	0.21	0.56			
Detected/sampled	13/13	14/14	12/14	10/10	12/12	12/12			
Enterobacteriaceae	9.20	8.33	6.97	9.05	8.34	7.75	0.157	<0.0001	0.137
SEM	0.10	0.09	0.27	0.11	0.10	0.29			
Detected/sampled	14/14	14/14	14/14	12/12	12/12	11/12			
Total Aerobes	9.32	8.64	8.41	9.28	8.42	8.24	0.026	<0.0001	0.654
SEM	0.09	0.09	0.09	0.10	0.10	0.10			
Detected/sampled	14/14	14/14	14/14	12/12	12/12	12/12			
Total Anaerobes	9.68^{x}	9.61^{x}	9.27^{z}	9.61^{x}	9.76^{y}	9.18^{z}	0.989	<0.0001	0.033
SEM	0.08	0.06	0.07	0.08	0.07	0.08			
Detected/sampled	14/14	14/14	14/14	12/12	12/12	12/12			

[1] A total of 26 litters were used in a nursing trial with 14 litter for control and 12 litter for Calsporin treatment. Pigs were given one daily oral dose of control or Calsporin from d 2 until d 19 (weaning), corresponding to d 0 until 17 of treatment, respectively. Calsporin dosage was 45×10^6 CFU/mL, 77.5×10^6 CFU/mL and 108.3×10^6 CFU/mL for d 0 to 7, d 7 to 14 and d 14 to wean, respectively. Fecal samples were collected directly from the rectum of the piglets on d 0, 7 and 14 after the beginning of treatment. Values are mean ± standard error of the mean. Units are $\log_{10}$ CFU/g. The superscripts ^{x,y,z} indicate significant difference among days within each treatment and ^{a,b} indicate significant difference between treatments within each day.

[2] Calsporin (Calpis Co. Ltd., Tokyo, Japan) is a direct-fed microbial based on viable spores of *Bacillus subtilis* C-3102.

[3] Limit of detection for Calsporin and total *Bacillus* sp. was 2×10^2 CFU/g.