Annals of Warsaw University of Life Sciences – SGGW Animal Science No 57 (4), 2018: 407–417 (Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 57 (4), 2018) DOI 10.22630/AAS.2018.57.4.39

SiO₂ nanostructures as a feed additive to prevent bacterial infections in piglets

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Abstract: SiO_2 nanostructures as a feed additive to prevent bacterial infections in piglets. The aim of the study was to determine the effect of a feed additive containing SiO₂ nanostructures (nSiO₂) mixed with organic acids encapsulated in a lipid matrix, fructooligosaccharide and Yucca Schidigera extract, on the prevalence of bacterial gastrointestinal infections in piglets, production parameters, nitrogen emission, and the condition of the sows during pregnancy. The experiment was carried out on 18 sows (Polish Landrace × Polish Large White) and 194 piglets from their litters, randomly divided into 3 groups (6 sows with litters each): experimental groups A and B fed standard diets supplemented with the additive differentiated by the concentration of nSiO₂ and control group fed standard diets without any additive. The additive was given to sows from the 100th day of pregnancy to the end of lactation, while to piglets from 7 to 70 days of age. The feed additive used significantly improved production parameters of weaned piglets, including body weight gain and feed intake ($P \le 0.05$). The analysis of gut microbiota showed a significant increase in the number of lactic acid bacteria and a decrease in the number of bacterial pathogens ($P \le$ 0.05), followed by reduced prevalence of diarrhea and ammonia emission ($P \le 0.05$) in groups A and B compared to control. The improved performance and prevention of bacterial diarrhea indicate the reasonable use of the feed additive in both tested doses in rearing of pigs.

Key words: SiO₂ nanostructures, gut microbiota, piglets, feed additives

INTRODUCTION

The rearing of piglets is one of the most difficult stages in the pig production cycle, often associated with gastrointestinal (GI) diseases and piglets losses. To reduce the occurrence of bacterial infections in piglets, it is necessary to develop innovative feed additives with multidirectional effects that will mimic the action of antibiotic growth promoters (AGP). The most popular feed additives with antibacterial properties include acidifiers that inhibit the growth of pHsensitive pathogens by lowering their internal pH (Ahmed et al. 2014). In turn, prebiotics such as fructooligosaccharides (FOS), promote the growth of commensal bacteria (lactic acid bacteria, Bifidobacterium) ensuring the proper composition of the GI ecosystem (Mikkelsen and Jensen 2004). Plant extracts, e.g. Yucca Schidigera with high amount of saponins, may further facilitate the bacteria penetration

by active compounds by reducing the surface tension of cells (Cheeke 2000). Other, less popular compounds, such as SiO₂ nanostructures (nSiO₂), due to their specific physicochemical properties, may also show strong bactericidal and toxin binding abilities (Pietroiusti et al. 2016).

It was assumed that the use of a feed additive containing a mixture of biologically active compounds through their synergistic bactericidal action will be effective in regulating intestinal microenvironment and improving piglets performance. Therefore, the aim of the study was to determine the effect of the feed additive containing organic acids encapsulated in a lipid matrix which allows for their gradual release in the entire GI tract, FOS, *Yucca Schidigera* extract and nSiO₂ on piglets survival and growth performance during rearing

period, gut microbiota composition and a prevalence of bacterial diarrhea, as well as nitrogen emission and sows condition during pregnancy.

MATERIALS AND METHODS

The experiment was conducted on 18 multiparous sows (Polish Landrace × Polish Large White) and 194 piglets from their litters, randomly divided into 3 groups (6 sows with their litters each): experimental groups A and B fed standard diets supplemented with the additive differentiated only by the concentration of nSiO₂, and control group fed the same diets but without any additive (Table 1). The additive contained fumaric, citric, malic and sorbic acids (POCh, Poland) encapsulated in a lipid matrix, FOS (Orafti P95, Beneo, Belgium), *Yucca Schidigera* extract (BioSol-YS-30S,

TABLE 1. Study scheme and feed additive composition

Item	Gestating sows			Lactating sows			
Ingredient (g/t of feed)	control	A	В	control	A	В	
nSiO ₂	_	500	1000	-	500	1000	
Organic acids*	_	600	600	-	800	800	
FOS	_	2000	2000	-	400	400	
Yucca Sch.	_	1000	1000	-	1200	1200	

	Nursed piglets prestarter I		Weaned piglets prestarter II			Weaned piglets starter			
Ingredient (g/t of feed)	control	A	В	control	A	В	control	A	В
nSiO ₂	_	400	800	_	600	1200	_	500	1000
Organic acids*	_	2000	2000	-	1500	1500	-	1200	1200
FOS	_	2000	2000	-	3000	3000	_	6000	6000
Yucca Sch.	_	1200	1200	_	1000	1000	_	800	800

^{*}Mixture of organic acids encapsulated in lipid matrix: 200, 100, 100 and 100 g/kg of fumaric, sorbic, citric and malic acid, respectively

(A300, Evonik, Germany) with particle size of 5-10 nm and absorption area of 380 m²/g. The additive was given to sows from the 100th day of pregnancy to

Ultra Bio-Logics Inc, USA) and nSiO₂ the end of lactation, while to piglets from 7 to 70 days of age. The piglets were weaned on the 28th day of life. The composition and nutritional value of diets is presented in Table 2. Piglets

TABLE 2. Composition and nutritional value of diets

Ingredient (g/kg)	Sows	Nursed piglets prestarter I	Weaned piglets prestarter II	Weaned piglets starter
Barley	30.0	12.91	25.0	23.0
Triticale	25.0	_	_	_
Maize	20.0	20.0	15.0	20.0
Soybean meal 46%	16.0	5.00	5.00	19.0
Wheat	_	30.0	25.0	25.0
Wheat bran	5.08	_	5.20	5.21
Fodder chalk	1.00	_	_	1.00
Monocalcium phosphate	0.60	0.70	1.00	0.95
NaCl	0.45	0.26	0.30	0.35
HP300	_	10.0	9.00	_
Phytase	0.01	_	_	_
Vegatable oil	1.00	2.50	2.00	2.00
Milk powder	_	16.0	10.0	2.00
Calcium formate	_	1.50	1.10	_
L-Lys 98%	0.23	0.31	0.40	0.46
L-Thr 98%	0.03	0.07	0.12	0.15
DL-Met 99%	_	0.02	0.14	0.14
L-Trp 98%	_	0.03	0.04	0.04
Vitamins and minerals	0.50	0.50	0.50	0.50
Additive*	0.65 vs. 0.70	0.56 vs. 0.60	0.61 vs. 0.67	0.85 vs. 0.90
Composition:				
Metabolic energy, MJ/kgCrude protein, g/kg	13.0 161	13.9 212	13.4 192	13.1 179
- Crude protein, g/kg - Lys, g/kg	9.08	13.5	12.6	12.0
– Met+Cys, g/kg	5.42	7.18	7.61	7.21
- Thr, g/kg	5.91	8.52	8.03	7.71
- Trp, g/kg	1.91	2.76	2.72	2.55
- Ca, g/kg	6.88	8.66	7.77	7.90
- P, g/kg	5.17	5.92	6.45	6.01
– Na, g/kg	2.07	1.50	1.61	1.69

^{*} Mixture of organic acids encapsulated in a lipid matrix, FOS, Yucca Schidigera extract and nSiO2 according to group allocation

received diets: prestarter I (4–27 days of age), prestarter II (28–49 days of age) and starter (50–70 days of age).

During the experiment, the animals were monitored for body weight changes and feed intake as well as general health status, taking into account the number of days with diarrhea. Sows' body weight during lactation and number of piglets born alive was also recorded. At the end of the experiment (70 days of age), 6 piglets from each group (3 \circlearrowleft and 3 \circlearrowleft) were sacrificed by intraperitoneal administration of pentabarbiturate (Thiopental, Sandoz, Switzerland), and samples from the cecum and colon were collected and secured for microbiological analysis. The content of the various microorganisms was determined by generally applicable Koch plate dilution method on specific agar: total number of bacteria (MPA medium), lactic acid bacteria (Demeter medium), Salmonella and Shigella (SS medium), Bacteroides (Schaedler agar medium with blood, anaerobic conditions), Enterococcus (Mac-Conkey agar), and Clostridium perfringens (BBL Clostridium difficile Selective Agar). Escherichia coli was identified using titer method (bromocresol purple broth with lactose) and confirmed by culture on Endo agar. The incubation time for all bacteria was 24 h at 37°C. During the section, digesta from the stomach, duodenum, jejunum, caecum and colon was also collected to determine the pH, while in the cecum and colon digesta, the content of volatile fatty acids (VFA) was determined by gas chromatography. Analysis of the feed composition, dry matter and N-ammonium content in feces collected in the last week of the experiment was carried out using AOAC (2000) methods.

The data was statistically analyzed using one-way ANOVA or Kruskal-Wallis test for *non*-gaussian *distribution* followed by Tukey or Dunn test, respectively, for multiparous comparisons (GraphPad Prism 7.0, CA, USA). Differences were considered statistically significant at $P \le 0.05$.

RESULTS AND DISCUSSION

The average feed intake and body weight losses in sows during lactation were similar among the groups (6.3, 6.7 vs. 6.0 kg and 30.2, 31.6 vs. 26.7 kg, in groups A, B vs. control, respectively, P > 0.05). The piglets rearing and production parameters are shown in Tables 3 and 4, respectively. The use of the additive containing nSiO₂ reduced both, the piglets losses in the post-weaning period and the diarrhea incidence by an average of 70% in both experimental groups A and B compared to control (Table 3). There was a significant increase and an upward trend in body weight gain of piglets in both pre- and post-weaning period in groups B and A, respectively, compared to control (Table 4). Feed intake throughout the study period was also significantly higher in groups A and B compared to control ($P \le 0.05$).

The obtained results are consistent with other studies where the use of acidifiers as well as a mixture of organic acids and probiotics positively influenced feed intake and rearing parameters in piglets (Rekiel and Kulisiewicz 1996, Janik and Pieszka 2006). It has been for example shown that diet supplementation with a mixture of organic acids during post-weaning period resulted in better gain and feed efficiency than pigs fed the con-

TABLE 3. Rearing parameters

Item	Group				
Item	6 6 63 65 63 2 (3.17%) 2 (3.08%) 2 (3.08%)	В			
Number of litters	6	6	6		
Number of piglets born alive	63	65	66		
Piglets losses between birth and weaning	2 (3.17%)	2 (3.08%)	2 (3.03%)		
Piglets losses between weaning and 70 days of age	3 (4.92%)	0	0		
Diarrhea incidence (number of piglets × number of days with diarrhea)	96 (2.18%)	34 (0.75%)	24 (0.52%)		

TABLE 4. Production parameters of piglets

T.		P						
Item	control	A	В	P				
		Body weigh	nt (kg)					
Day 1 of age	1.58 ±0.04	1.60 ±0.04	1.68 ±0.04	0.121				
Day 70 of age	18.9 ±1.12 ^A	22.7 ±1.28 ^B	24.5 ±1.34 ^A	0.001				
		ADG (g/day)						
1–28 days of age	198 ±15a	224 ±17 ^b	238 ±21a	0.028				
28–42 days of age	124 ±7a	140 ±9 ^b	178 ±10a	0.024				
42-70 days of age	370 ±18ab	482 ±20a	487 ±21 ^b	0.011				
1-70 days of age	246 ±11 ^A	296 ±15 ^B	333 ±16 ^A	0.009				
	ADFI (g/day/piglet)							
1–28 days of age	27	27	32	0.422				
28–42 days of age	220	231	236	0.766				
42-70 days of age	579ª	654 ^{bc}	706 ^{ac}	0.011				
1–70 days of age	348ac	396ª	415°	0.039				
	FCR (kg/kg)							
1–28 days of age	0.14	0.13	0.10	0.597				
28-42 days of age	1.48	1.54	1.71	0.495				
1–70 days of age	1.22	1.18	1.17	0.153				

A, B – means in the rows with the same capital letters differ significantly at $P \le 0.01$. a, b, c – means in the rows with the same small letters differ significantly at $P \le 0.05$. ADG – calculated as (finish body weigh-start body weight)/days. ADFI - calculated as feed intake per group/days/number of piglets in group. FCR - calculated as feed intake/weight gain.

trol diet (Li et al. 2008). Similarly, a blend of short chain and medium chain fatty acids as well as phenolic compounds

added to weaned pigs' diet also improved body weight gains and feed efficiency to comparable levels after using AGP (Long et al. 2018). In the same study a reduced incidence of diarrhea was also observed and it is know that post-weaning diarrhea can also strongly influence mortality and retardation of growth in piglets (Long et al. 2018). Acidifiers by lowering the pH in GI tract, enhance activity of proteolytic enzymes, slow down gastric retention time, and thus improve protein and amino acid metabolism and apparent total tract digestibility (Gerritsen et al. 2010, Ahmed et al. 2014). The growth promotional effects of these compounds, and thus the feed additive used in this study, might also result from direct suppression of pathogenic microbes in GI tract (Ahmed et al. 2014).

The results of intestinal microflora analysis are presented in Table 5. A significant increase in total bacteria and *Lactobacillus* and *Enterococcus* number

in both cecum and colon in groups A and B compared to control was observed $(P \le 0.01)$. Simultaneously, there was a significant decrease in the number of Enterobacteria, Salmonella and Shigella and Clostridium in groups A and B compared to control ($P \le 0.05$). The number of E. coli showed a downward trend in group B compared to control. The use of organic acids in feed additives stabilize the intestinal microflora not allowing the development of pathogenic bacteria by disrupting enzymes activity and signal transduction in pH-sensitive bacteria (Piva et al. 2007). A similar antimicrobial action against gut pathogens without harming beneficial bacteria was showed in piglets treated by a different mixtures of organic acids (Li et al. 2008, Øverland et al. 2008, Ahmed et al. 2014, Long et al. 2018).

TABLE 5. Composition of intestinal microflora (expressed as log cfu/g)

	Group		Р	Group			P	
Item	control	A	В	Р	control	A	В	Р
		Cec	um			Co	lon	
Total bacteria number	6.73 ±0.02 ^A	7.29 ±0.04 ^A	7.04 ±0.08 ^A	<0.0001	6.88 ±0.02 ^A	7.44 ±0.04 ^A	7.20 ±0.08 ^A	<0.0001
Lactobacillus/ Enterococcus	6.10 ±0.57 ^A	6.91 ±0.06 ^A	6.69 ±0.04 ^B	<0.0001	6.25 ±0.57 ^A	7.06 ±0.06 ^A	6.84 ±0.04 ^B	<0.0001
Enterobacteria	6.65 ±0.20 ^A	6.22 ±0.14 ^A	5.89 ±0.08 ^A	< 0.0001	6.65 ±0.20 ^A	6.22 ±0.14 ^A	5.89 ±0.08 ^A	<0.0001
Salmonella and Shigella	2.35 ±0.41 ^{ab}	0.98 ±0.87a	0.80 ±0.95 ^b	0.0049	2.34 ±0.59	1.08 ±0.94	0.92 ±1.05	0.0221
Bacteroides/ Prevotella	4.00 ±0.06 ^A	3.10 ±0.18 ^{AB}	4.13 ±0.09 ^B	<0.0001	4.16 ±0.06 ^A	3.26 ±0.18 ^{AB}	4.29 ±0.09 ^B	<0.0001
Clostridium	3.65 ±0.29 ^{aB}	1.92 ±0.98 ^a	1.00 ±1.55 ^B	0.0004	3.76 ±0.28 ^{aB}	2.12 ±1.08 ^a	1.07 ±1.66 ^B	0.0009
Escherichia coli (cfu/mL)	2.19×10^{-7}	3.67 × 10 ⁻⁷	5.35×10^{-8}	0.7737	2.19×10^{-5}	3.67 × 10 ⁻⁵	5.35 × 10 ⁻⁶	0.8941

A, B – means in the rows with the same capital letters differ significantly at $P \le 0.01$. a, b – means in the rows with the same small letters differ significantly at $P \le 0.05$.

Prebiotics such as FOS which are not digested in small intestine, may serve as the substrate for commensal gut microflora and stimulate their growth. Mikkelsen and Jensen (2004) showed that diet supplementation with FOS result in increased number of Lactobacillus and reduced number of E. coli in GI tract of piglets. In turn, the antibacterial effects of nSiO2 are not fully understood but they might be related to their specific electrostatic interactions that modify electric charge on the surface of the bacterial membrane and disrupt their integrity (Thill et al. 2006, Hajipour et al. 2012). The second possible mechanism implies the increased production of free radicals and induction of oxidative stress what have a strong adverse effect on membranes and other cell structures (Soenen et al. 2011). The reports on the antibacterial effect of silica nanoparticles in animals are very limited, however several studies showed that natural and synthetic zeolites are very effective in prevention of bacterial infections in farm animals (Papaioannou et al. 2005), and on the other hand, engineered nanomaterials might have a positive effect on the composition of GI microflora and animals' growth, however the effect is strongly dependent on the individual properties of nanoparticles used (e.g. particles size) as well as dose and time of administration (Pietroiusti et al. 2016).

For example, a study by Fondevila et al. (2009) showed that the addition of silver nanoparticles (size of 60–100 nm) in diets for weanling pigs led to improved growth parameters in a dose dependent manner and reduced ileal concentration of coliforms, total bacteria, *Clostridium* and *Atopobium* without affecting the lactobacilli content.

An exposure of the same animal model to copper-loaded nanoparticles also resulted in increased average daily gain and feed intake and decreased diarrhea rate, as well as favorable changes in intestinal bacteria content with increasing amount of lactobacillus and bifidobacterium and reduced number of E. coli (Wang et al. 2012). Another study performed in mice showed that oral exposure to 5-500 ppm of silica nanoparticles or to 46-4600 pbb of silver nanoparticles mixed in food may also affect the gut microbiota (Lecloux et al. 2015). Moreover, several classes of antimicrobial nanoparticles have proven their effectiveness for treating infectious diseases, including antibiotics resistant one (Hajipour et al. 2012).

It should be however noted that nanoparticles might show cyto- and immunotoxic effects through the direct effect on gut microbiota or through systemic effects of metabolites generate (Xu et al. 2010, Pietroiusti et al. 2016). In study on mice it has been showed that nano and micron sized silica (30 nm and 30 µm, respectively) induce similar biological effect, however diet supplementation with nanosilica results in an elevated plasma ALT level suggesting its effect hepatotoxic, even though there were no differences in general health of mice after feeding of 140 g silica/kg body weight (So et al. 2008). A risk assessment studied of synthetic amorphous silica in food (<100 nm, food additive E551) showed that it is characterized by low GI absorption and it may pose a health risk through liver accumulation but the results are not conclusive (van Kesteren et al. 2014). All these suggest that nanoparticles might be an interesting alternative to AGP used in animals nutrition however more detailed

studies are still needed to determine their specific mode of action and all possible side effects should be taken into consideration.

The data on gastrointestinal pH profile, VFA, ammonia and dry matter content are shown in Table 6. The use of the additive resulted in increased VFA content in cecum and distal part of colon, as well as reduced ammonia content in cecum and feces in both experimental groups A

and B compared to control ($P \le 0.05$). Ammonium ions adversely affect the gut environment creating alkaline conditions that are more favorable for the invasion of pathogenic bacteria (Papaioannou et al. 2005). Moreover, exposure to ammonia malodor is an environmental stressor for both humans and animals as well as an important source of air pollution (Cambra-Lopez et al. 2010). The reduction in intestinal ammonia formation

TABLE 6. Gastrointestinal pH profile, VFA, ammonia and dry matter content

Item		P							
nem	control	A	В						
pН									
Stomach	3.97 ±0.04	3.94 ±0.03	3.92 ±0.03	0.18					
Duodenum	6.02 ±0.06	5.93 ±0.05	5.91 ±0.04	0.11					
Jejunum	6.55 ±0.08	6.40 ±0.09	6.38 ±0.09	0.06					
Cecum	6.00 ±0.05	5.96 ±0.06	5.92 ±0.07	0.12					
Colon	5.98 ±0.07	6.09 ±0.09	6.14 ±0.10	0.07					
		/FA (mmol/kg)							
Cecum	80.2 ±7.6ab	111 ±8.8a	113 ±9.1 ^b	0.048					
Colon	90.9 ±4.5 ^{AB}	149 ±5.8 ^A	152 ±5.9 ^B	0.009					
	VFA, molar	proportions in colon	(%)						
Acetic acid	63.1 ±7.06 ^{AB}	50.0 ±5.27 ^A	49.0 ±5.41 ^B	0.01					
Propionic acid	23.8 ±2.34	28.6 ±2.75	29.0 ±2.90	0.24					
Butyric acid	9.95 ±0.25ab	16.4 ±0.28 ^a	17.1 ±0.30 ^b	0.039					
Isobutyric acid	0.45 ±0.03ab	1.24 ±0.04 ^a	1.20 ±0.04b	0.028					
Valeric acid	2.01 ±0.09ab	2.76 ±0.11 ^a	2.62 ±0.10 ^b	0.046					
Isovaleric acid	0.69 ±0.06ab	0.91 ±0.07a	1.07 ±0.08 ^b	0.012					
	Ammon	ia (mg/g of dry matter))						
Cecum	0.72 ±0.07ab	0.45 ±0.05 ^b	0.41 ±0.05 ^a	0.048					
Feces	1.06 ±0.07 ^{ab}	0.56 ±0.06a	0.50 ±0.05 ^b	0.011					
Dry matter (%)									
Cecum	15.0 ±0.02	15.6 ±0.03	16.2 ±0.04	0.52					
Feces	27.0 ±0.17	26.2 ±0.16	26.4 ±0.15	0.74					

A, B – means in the rows with the same capital letters differ significantly at $P \le 0.01$. a, b – means in the rows with the same small letters differ significantly at $P \le 0.05$.

have been also reported by others with the use of organic acid, prebiotics, natural and synthetic zeolites as well as Yucca Schidigera extracts (Yen and Pond 1990, Papaioannou et al. 2005, Windisch et al. 2008). This may result from the stimulation of intestinal microflora activity which uses the nitrogen for a synthesize of its own proteins. Similarly, prebiotics and selected organic acids may facilitate the production of VFA in piglets GI tract (Van Loo et al. 1999, Suiryanrayna and Ramana 2015).

CONCLUSIONS

The use of additive with multidirectional activity containing a mixture of nSiO₂, organic acids encapsulated in a lipid matrix, FOS and Yucca Schidigera extract in the feed ratios for piglets positively influence production and rearing parameters by increasing ADG and ADFI, as well as reducing piglets losses, diarrhea incidence and nitrogen emission. The additive used improved intestinal microflora composition by favoring the growth of commensal bacteria and limiting the growth of pathogens. The tested additive is an interesting and effective alternative for AGP in the prevention of bacterial infections and promotion of health and growth in young animals, and its use in both tested nSiO₂ doses seems to be reasonable during rearing period of piglets.

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Streszczenie: Nanostruktury SiO2 jako dodatek paszowy zapobiegający infekcjom bakteryjnym u prosiąt. Celem badań było określenie wpływu dodatku paszowego zawierającego nanostruktury SiO₂ (nSiO₂) wraz z kwasami organicznymi chronionymi w matrycy lipidowej, fruktooligosacharydem i ekstraktem z Yucca Schidigera na częstość występowania infekcji bakteryjnych przewodu pokarmowego u prosiąt, parametry produkcyjne, ilość emitowanego azotu oraz kondycję loch w czasie ciąży. Doświadczenie przeprowadzono na 18 lochach (pbz x wbp) oraz 194 prosiętach pochodzących z ich miotów, przydzielonych losowo do 3 grup (6 loch wraz z miotami każda): Grupy doświadczalne A i B otrzymujące standardowe pasze wzbogacone o badany dodatek zróżnicowany jedynie pod względem zawartości nSiO2, oraz grupę kontrolną otrzymującą standardową paszę bez dodatku. Dodatki podawano lochom od 100. dnia ciąży do końca okresu laktacji, prosiętom natomiast od 7. do 70. dnia życia. Zastosowane dodatki paszowe miały istotny wpływ na poprawę parametrów odchowu odsadzonych prosiąt, w tym przyrosty masy ciała i pobranie paszy ($P \le 0.05$). Analiza mikrobiologiczna treści pokarmowej jelit wykazała istotny wzrost liczby bakterii kwasu mlekowego i spadek liczebności bakterii patogennych w grupach A i B w porównaniu do kontroli ($P \le 0.05$), ograniczając częstość występowania biegunek oraz emisję azotu $(P \le 0.05)$. Poprawa wydajności oraz ograniczenie występowania biegunek bakteryjnych wskazują na uzasadnione zastosowanie badanego dodatku w obu testowanych dawkach w czasie odchowu prosiąt.

Słowa kluczowe: nanostruktury SiO2, mikroflora jelitowa, prosięta, dodatki paszowe

MS received 16.03.2018 MS accepted 12.09.2018

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