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Effect of supplementation of nanochitosan on the growth performance, small intestinal morphology and gut pathogen load in crossbred (LWY x DESI) piglets

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Abstract

In a completely randomized design, the effect of nanochitosan from chitosan as an alternative to antibiotics was evaluated using 4 liters per treatment such that the four dietary treatments had 32 piglets in each group. The dietary treatments were creep feed (T1, control), creep feed with antibiotic (T2), creep feed with 1.0% chitosan (T3), creep feed with 0.1% nanochitosan (T4) supplementation. The piglets were weaned at 42 days and fed for another 14 days by selecting at random 6 piglets for each treatment. Grower ration (T1) was supplemented with antibiotic (T2), 5.0% chitosan (T3), 0.5% nanochitosan (T4). During pre-weaning phase, the initial piglet weight (kg), final piglet weight (kg), average daily gain and weight gain were significantly (P<0.01) different among treatments. During grower phase, the total weight gain (kg) and average daily gain (kg) of pigs were higher (P<0.01) in T3 and T4. At 10th day, the villi height (µm), crypt depth (µm) and ratio of villus height to crypt depth (VH/CD) in duodenum, jejunum and ileum and the *E. coli* and *Salmonella* count (cfu/g) was not significantly different among treatments T1 to T4. At 42nd day, the villi height (µm) in duodenum was significantly higher (P<0.01) in pigs fed on T4 ration but in jejunum and ileum, height of villi (µm) was significantly higher (P<0.01) in pigs fed on T3 and T4 rations. The crypt depth (µm) in duodenum was significantly lower (P<0.01) in pigs fed on T2 ration, whereas, in jejunum and ileum the crypt depth (µm) was significantly lower (P<0.01) in pigs fed on T3 and T4. The ratio of villus height to crypt depth (VH/CD) in duodenum, jejunum and ileum was significantly higher (P<0.01) in pigs fed on T4 ration. The *E. coli* (P<0.01) and *Salmonella* (P<0.05) count in pigs fed on T4 ration was significantly lower. At 57th day, the height of villi (µm) and ratio of villus height to crypt depth (VH/CD) in duodenum, jejunum and ileum in pigs fed on T4 ration was significantly higher (P<0.01). The crypt depth (µm) in duodenum, jejunum and ileum was significantly lower (P<0.01) in pigs fed on T4 ration than in other treatments. The *E. coli* and *Salmonella* count (cfu/g) was significantly lower (P<0.01) in pigs fed on T3 and T4 rations.

Keywords: Nanochitosan, growth performance, intestinal morphology and gut pathogen load

Introduction

In India commercial Pig farming for pork production is increasing rapidly. Alterations in the intestinal structure and function, such as villous atrophy and crypt hyperplasia, which are generally associated with poor performance and temporary decrease in the digestive and absorptive capacity of the small intestine (Pluske *et al*. 1997) [1].

Antibiotics have been used to improve growth performance for many years. However antibiotic resistance may cause problems for human health issues which contribute to the spread of drug-resistant pathogens in both livestock and humans. Consumption of functional oligosaccharides such as chito-oligosaccharides can promote animal growth and development, enhance immune functions and improve animal microbial flora (Patterson and Burkholder, 2003) [2].

Nanochitosan can be used as feed additive to improve livestock production. Nano technology can be used in animal feeding to improve bioavailability of nutrients, production performance and immune status in livestock. Moreover chitosan is a very good adsorbent and the surface area of chitosan can be increased by converting it into nano chitosan (Govindarajan *et al*. 2011) [3].

Materials and Methods

The feed ingredients maize, soya bean meal and de-oiled rice bran were prepared with standard experimental diets in ground form procured from M/s Suvera Agencies, Chittoor. Chitosan was procured from Aura biotechnologies private limited, Chennai.

Representative samples of feed ingredients and experimental diets were analyzed for proximate composition (AOAC, 2005) [4]. 4 litters were assigned at random to 4 dietary treatments. The standard ration (T1) was supplemented with 0.05% oxymycin (T2) while chitosan was included at 1.0% (T3) and nanochitosan at 0.1% (T4). During the grower phase i.e., for a period of two weeks after weaning, 6 weaners per treatment were selected and fed with grower ration (T1), supplemented with 0.05% oxymycin (T2), 5.0% chitosan (T3), 0.5% nanochitosan (T4).

Two piglets per treatment at 10th day, 42nd day and 57th day were slaughtered to study the intestinal morphology and gut pathogen load. The data were analysed statistically by using one way analysis of variance.

Nanochitosan synthesis

Nano chitosan from chitosan was prepared by the modified ion-tropic gelation technique used by Vellingiri *et al.* (2013) [5]. The Chitosan solution 5mg/ml was prepared by dissolving the polymer in 1.5% (w/v) acetic acid aqueous solution for 0.5 hours under magnetic stirring. A 0.5% w/v concentration of sodium tripolyphosphate (STPP) solution was also prepared with distilled water. The STPP solution was added drop wise with a syringe to chitosan solution while stirring, this will leads to the formation of chitosan nanoparticles by interaction between chitosan and sodium tripolyphosphate.

Study of small intestine morphology

The small intestine was excised immediately after slaughter and approximately 3 cm segments of the duodenum, jejunum and ileum were taken from the middle of each part, cut transversely, washed with 0.9% NaCl and immersed in 10% buffered formalin for fixation.

Samples from the formalin preserved segments were cut into approximately 3 - 5 µm thickness and stained with haematoxylin and eosin (Prophet *et al*. 1992) [6]. Well oriented crypt-villus units were selected for each intestinal cross-section and villus height and the crypt depth were measured at 10 X magnification using a light microscope equipped with an ocular micrometer (Li *et al*. 1990) [7] and the ratio of villus to crypt depth was calculated.

Collection of gut pathogens

A portion of large intestine between caecum and colon measuring 10 cm length was ligated on both the sides and it was cut behind the ligation on the both the sides with a sterilized knife. The collected intestine piece was placed in a beaker containing normal saline solution to estimate gut pathogens. The data were subjected to one-way analysis of variance and the means were tested by least significant difference.

Results and Discussion

Growth performance

During, the pre-weaning phase (Table 1), the initial litter weights (kg) were significantly lower (P<0.01) in T4 whereas at the end of pre-weaning phase (42 days) the litter weights were significantly higher (P<0.01) in T3 and T4 than in T1 and T2. The total litter weight gain (kg) and average daily gain of litter (kg) was significantly higher (P<0.01) in T4 than in other treatments. There was no significant difference among treatments in total creep feed intake (kg). The feed intake per kg wt gain was significantly lower (P<0.01) in T4 than in other treatments. These results were similar to the earlier studies of Xu *et al*. (2013 and 2014) [8, 9], Swiatkiewicz *et al.* (2015) [10] and Suthongsa *et al.* (2017) [11].

During grower phase (Table 2), the initial body weights (kg) were not significantly different among the treatments whereas at the end of grower phase the body weights of the pigs and average daily feed intake in T4 were significantly higher (P<0.01) than the other treatments. The total weight gain of pigs (kg) in T1 was significantly lower (P<0.01) than in other treatments. The average daily gain of pigs (kg) in T3 and T4 was significantly higher (P<0.01) than in other treatments. The feed intake per kg wt gain was significantly higher (P<0.01) in T1 than in other treatments. The results of this study demonstrated that diets supplemented with chitosan promoted better growth performance of weaned piglets, which was in agreement with the Xu *et al*. (2013) [8].

In the current study, feed intake was increased by dietary supplementation of chitosan, which may have contributed to the increased ADG of pigs which was in agreement with Suthongsa *et al.* (2017) [11].

Small intestine morphology

At 10th day

The villi height (µm), crypt depth (µm) and ratio of villus height to crypt depth (VH/CD) in duodenum, jejunum and ileum were not significantly different among the treatment groups.

At 42nd day

In the present study, the villi height (µm) in duodenum was significantly higher (P<0.01) in pigs fed with T4 than in T1 and T2. The crypt depth (µm) in duodenum was significantly lower (P<0.01) in pigs fed with T2 than in other treatments. The ratio of villus height to crypt depth (VH/CD) in duodenum was also significantly higher (P<0.01) in T4 fed pigs than in other treatments.

The height of villi (µm) and ratio of villus height to crypt depth (VH/CD) in jejunum was significantly higher (P<0.01) in pigs fed with T3 and T4 fed pigs than in T1 and T2. The crypt depth (µm) in jejunum was significantly lower (P<0.01) in pigs fed with T3 and T4 and comparable in pigs fed T1 and T2.

The villi height (µm) in ileum of pigs fed T3 and T4 was significantly higher (P<0.01) than in pigs fed with T1 and T2. The crypt depth (µm) in ileum was significantly lower (P<0.01) in pigs fed with T3 and T4 and comparable in pigs fed T1 and T2. The ratio of villus height to crypt depth (VH/CD) in ileum was significantly higher (P<0.01) in T4 fed pigs and comparable in T1 and T2.

The feeding of chitosan has positive effect in improving the morphological structure of small intestine which has in agreement with the findings of Xiao *et al.* (2013) [12], Xu *et al*. (2013) [8], Suthongsa *et al.* (2017) [11], Wan *et al.* (2017) [13] and Yue *et al*. (2017) [14]. Maximum digestion and absorption occurred as the villus: crypt ratio increased (Pluske *et al*. 1996) [15] in newly weaned pigs.

At two weeks after weaning (57th day)

The villi height (µm) and ratio of villus height to crypt depth (VH/CD) in duodenum, jejunum and ileum of pigs fed on T4 ration was significantly higher (P<0.01) than that of other treatments. The crypt depth (µm) in duodenum, jejunum and ileum was significantly lower (P<0.01) in pigs fed on T4 than in other treatments which was the major criteria to evaluate small intestine maturity in pigs. The findings are in agreement with those of Klemm and Schembri (2000) [16].

The percent increase of villus height from 42nd day to 57th day was higher in T4 than in other treatments and the values were 6.48%, 7.67%, 9.01% and 10.43% (duodenum); 6.95%, 9.24%, 8.94%, 9.72% (jejunum) and 8.38%, 9.96%, 8.74% and 11.36% (ileum) for T1 to T4 fed pigs respectively.

The percent decrease of crypt depth from 42nd day to 57th day was higher in T4 than in other treatments and the values were 13.69%, 9.68%, 24% and 26.18% (duodenum); 7.84%, 6.74%, 10%, 12.47% (jejunum) and 5.6%, 6.16%, 8.3% and 9.83% (ileum) for T1 to T4 fed pigs respectively.

The percent increase in ratio of villi height to crypt depth from 42nd day to 57th day was higher in T3 and T4 than in T1 and T2 and the values were 22.58%, 20%, 41.54% and 30.48% (duodenum); 15.68%, 17.42%, 20.98% and 25.19 (jejunum) and 15.78%, 17.3%, 18.32% and 24.13% (ileum) for T1 to T4 fed pigs respectively.

The supplementation of nano chitosan followed by chitosan was beneficial in improving intestinal morphology as indicated by the percent increase in villi height, villi height to crypt depth ratio and decrease in crypt depth clearly indicating their superiority over antibiotic feed supplement in overcoming weaning stress.

Gut pathogen load

At 10th day, the *E. coli* and *Salmonella* count (cfu/g) was not significantly different among treatments T1 to T4.

At 42nd day (weaning), the *E. coli* count in pigs fed on T4 ration was significantly lower ($P<0.01$), while, the *Salmonella* count in pigs fed on T1 ration was significantly higher ($P<0.05$), than the other treatments which are in agreement with Xu *et al.* (2013) [8] and Han *et al.* (2012) [17].

At 57th day (two weeks after weaning), the *E. coli* and *Salmonella* count (cfu/g) was reduced significantly ($P<0.01$) in T3 and T4 rations. The percent decrease in *E. coli* count from 10th day to 57th day was higher in T4 than in other treatments. The percent decrease in *Salmonella* count from 10th day to 57th day was higher in T3 and T4 than in T1 and T2.

Table 1: Effect of experimental diets on growth performance of crossbred pigs during pre-weaning phase (10-42 days)

Parameter	T1	T2	T3	T4
Initial weight of piglet at 10th day (kg)** n = 32	$2.48^{b} \pm 0.16$	$1.80^{a} \pm 0.08$	$2.21^{b} \pm 0.08$	$1.70^{a} \pm 0.07$
Final weight of piglet at 42nd day (kg)** n = 32	$6.76^{ab} \pm 0.37$	$6.13^{a} \pm 0.33$	$7.32^{b} \pm 0.33$	$7.45^{b} \pm 0.33$
Weight gain/piglet (kg)** (n = 32)	$4.28^{a} \pm 0.24$	$4.33^{a} \pm 0.27$	$5.10^{ab} \pm 0.26$	$5.74^{b} \pm 0.27$
No. of days	32	32	32	32
Average daily gain/litter (kg)** (n = 4)	$1.06^{a} \pm 0.01$	$1.08^{ab} \pm 0.05$	$1.27^{bc} \pm 0.05$	$1.43^{c} \pm 0.04$
Total feed intake/litter (kg)^{NS} (n = 4)	35.37 ± 1.46	34.65 ± 1.16	33.85 ± 1.14	31.54 ± 0.74
Feed/kg gain**	$1.03^{b} \pm 0.05$	$1.00^{b} \pm 0.06$	$0.83^{ab} \pm 0.04$	$0.68^{a} \pm 0.03$

^{abcd} Values in a row not sharing common superscripts differ significantly **($P<0.01$)

^{NS} Not significant

Table 2: Effect of experimental diets on growth performance of crossbred pigs during grower phase (Up to two weeks after weaning)

Parameter	T1	T2	T3	T4
Initial weight (kg)^{NS} (n = 6)	10.42 ± 0.43	10.55 ± 0.35	11.29 ± 0.34	11.64 ± 0.46
Final weight (kg)** (n = 6)	$12.52^{a} \pm 0.35$	$13.25^{ab} \pm 0.42$	$14.78^{bc} \pm 0.32$	$15.62^{c} \pm 0.43$
Weight gain (kg)**	$2.05^{a} \pm 0.07$	$2.69^{a} \pm 0.25$	$3.49^{b} \pm 0.15$	$3.98^{b} \pm 0.12$
No. of days	14	14	14	14
Average daily gain (kg)**	$0.14^{a} \pm 0.00$	$0.18^{b} \pm 0.17$	$0.24^{c} \pm 0.01$	$0.28^{c} \pm 0.00$
ADFI (kg)**	$0.20^{a} \pm 0.00$	$0.24^{ab} \pm 0.01$	$0.27^{bc} \pm 0.00$	$0.30^{c} \pm 0.01$
Feed/kg gain **	$1.48^{b} \pm 0.06$	$1.35^{ab} \pm 0.15$	$1.12^{ab} \pm 0.06$	$1.06^{a} \pm 0.03$

^{abcd} Values in a row not sharing common superscripts differ significantly ** ($P<0.01$), ^{NS} Not significant

Table 3: Intestinal morphological changes among experimental groups at 10th day

Parameter		Treatments			
		T1	T2	T3	T4
Duodenum	Villus height (µm)	49.80 ± 1.15	52.20 ± 2.22	49.80 ± 1.65	50.40 ± 1.96
	Crypt depth (µm)	43.20 ± 1.74	43.40 ± 1.91	46.20 ± 2.37	48.40 ± 1.6
	VH/CD2	1.15 ± 0.03	1.2 ± 0.06	1.07 ± 0.02	1.04 ± 0.03
Jejunum	Villus height (µm)	60.40 ± 1.56	59.40 ± 2.01	60.80 ± 1.39	61.00 ± 1.14
	Crypt depth (µm)	53.80 ± 3.45	55.20 ± 2.59	52.60 ± 3.12	53.20 ± 3.10
	VH/CD2	1.13 ± 0.16	1.08 ± 0.19	1.17 ± 0.18	1.16 ± 0.2
Ileum	Villus height (µm)	54.80 ± 1.39	55.60 ± 1.2	56.20 ± 1.42	56.80 ± 2.39
	Crypt depth (µm)	49.00 ± 2.28	51.40 ± 1.77	48.80 ± 1.56	49.80 ± 2.72
	VH/CD2	1.12 ± 0.05	1.08 ± 0.04	1.15 ± 0.04	1.14 ± 0.07

1 Mean of four observations, 2 VH/CD means the ratio of villus height to crypt depth.

Table 4: Intestinal morphological changes among experimental groups at 42th day

Parameter		Treatments			
		T1	T2	T3	T4
Duodenum	Villus height (µm)	$163.40^{a} \pm 2.33$	$169.40^{a} \pm 2.65$	$255.00^{b} \pm 1.94$	$276.00^{c} \pm 6.65$
	Crypt depth (µm)	$175.20^{bc} \pm 2.63$	$152.80^{a} \pm 4.15$	$178.80^{c} \pm 0.86$	$165.00^{ab} \pm 3.84$
	VH/CD2	$0.93^{a} \pm 0.00$	$1.10^{b} \pm 0.04$	$1.42^{c} \pm 0.03$	$1.66^{d} \pm 0.09$
	Villus height (µm)	$169.60^{a} \pm 1.56$	$235.80^{b} \pm 2.0$	$301.80^{c} \pm 5.93$	$314.60^{c} \pm 9.9$

Jejunum	Crypt depth (µm)	$165.80^{b} \pm 5.48$	$177.80^{b} \pm 1.93$	$124.00^{a} \pm 2.02$	$121.80^{a} \pm 1.06$
	VH/CD2	$1.02^{a} \pm 0.04$	$1.32^{b} \pm 0.01$	$2.43^{c} \pm 0.05$	$2.58^{c} \pm 0.08$
Ileum	Villus height (µm)	$164.60^{a} \pm 4.83$	$176.60^{a} \pm 1.86$	$258.40^{b} \pm 5.62$	$271.00^{b} \pm 5.64$
	Crypt depth (µm)	$171.40^{b} \pm 1.16$	$168.80^{b} \pm 2.13$	$134.80^{a} \pm 2.65$	$132.20^{a} \pm 2.47$
	VH/CD2	$0.95^{a} \pm 0.02$	$1.04^{a} \pm 0.01$	$1.91^{b} \pm 0.03$	$2.03^{c} \pm 0.02$

1 Mean of four observations, 2 VH/CD means the ratio of villus height to crypt depth.

Table 5: Effect of experimental diets on small intestinal morphology at 57th day

Parameter		Treatments			
		T1	**T2**	**T3**	**T4**
Duodenum	Villus height (µm)	$174.00^{a} \pm 3.00$	$182.40^{a} \pm 2.20$	$278.00^{b} \pm 6.42$	$304.80^{c} \pm 6.44$
	Crypt depth (µm)	$151.20^{c} \pm 2.15$	$138.00^{b} \pm 3.42$	$135.80^{b} \pm 2.61$	$121.80^{a} \pm 3.48$
	VH/CD2	$1.14^{a} \pm 0.03$	$1.32^{a} \pm 0.02$	$2.01^{b} \pm 0.04$	$2.50^{c} \pm 0.12$
Jejunum	Villus height (µm)	$181.40^{a} \pm 2.15$	$257.60^{b} \pm 1.74$	$328.80^{c} \pm 1.93$	$345.20^{d} \pm 3.12$
	Crypt depth (µm)	$152.80^{b} \pm 4.15$	$165.80^{b} \pm 4.75$	$111.60^{a} \pm 1.36$	$106.60^{a} \pm 1.28$
	VH/CD2	$1.18^{a} \pm 0.03$	$1.55^{b} \pm 0.03$	$2.94^{c} \pm 0.04$	$3.23^{d} \pm 0.03$
Ileum	Villus height (µm)	$178.40^{a} \pm 1.50$	$194.20^{a} \pm 3.55$	$281.00^{b} \pm 4.88$	$301.80^{c} \pm 5.26$
	Crypt depth (µm)	$161.80^{b} \pm 4.57$	$158.40^{b} \pm 2.99$	$123.60^{a} \pm 1.56$	$119.20^{a} \pm 1.56$
	VH/CD2	$1.10^{a} \pm 0.02$	$1.22^{a} \pm 0.04$	$2.26^{b} \pm 0.03$	$2.52^{c} \pm 0.03$

The number of observations for each mean value was four (n = 4), VH/CD = The ratio of villus height to crypt depth, [abcd] Values in a row not sharing common superscripts differ significantly (P<0.01)

Table 6: The effect of experimental diets on intestinal morphology from 42nd day to 57th day

Parameter		Treatments			
		T1	**T2**	**T3**	**T4**
Duodenum	Villus height (% increase)	6.48	7.67	9.01	10.43
	Crypt depth (% decrease)	13.69	9.68	24	26.18
	VH/CD2 (% increase)	22.58	20	41.54	30.48
Jejunum	Villus height (% increase)	6.95	9.24	8.94	9.72
	Crypt depth (% decrease)	7.84	6.74	10	12.47
	VH/CD2 (% increase)	15.68	17.42	20.98	25.19
Ileum	Villus height (% increase)	8.38	9.96	8.74	11.36
	Crypt depth (% decrease)	5.6	6.16	8.3	9.83
	VH/CD2 (% increase)	15.78	17.3	18.32	24.13

Table 7: Effect of experimental diets on gut pathogen load at different stages of growth

Pathogenic bacteria	T1	T2	T3	T4
At 10th day				
E. coli NS	92.50 ± 3.5	85.50 ± 1.5	96.50 ± 4.5	92.0 ± 1.0
Salmonella NS	104.00 ± 2.0	106.50 ± 1.5	109.00 ± 6.0	108.00 ± 1.0
At 42nd day				
*E. coli***	$76.50^{d} \pm 2.5$	$63.50^{bc} \pm 5.5$	$46.0^{ab} \pm 3.0$	$33.00^{a} \pm 3.0$
*Salmonella**	$67.00^{b} \pm 6.0$	$71.50^{b} \pm 2.5$	$58.50^{ab} \pm 3.5$	$45.50^{a} \pm 5.5$
At 57th day				
*E. coli***	$61.5^{c} \pm 2.5$	$50.50^{bc} \pm 2.5$	$37.50^{ab} \pm 2.12$	$20.0^{a} \pm 5.65$
Salmonella **	$51.50^{b} \pm 3.5$	$45.0^{ab} \pm 3.0$	$26.50^{a} \pm 3.5$	$27.0^{a} \pm 2.0$

[abcd] Values in a row not sharing common superscripts differ significantly *(P<0.05) **(P<0.01), NS Not significant

Table 8: Effect of experimental diets on gut pathogen load among different treatments between 10th day and 57th day

Pathogenic bacteria (% Decrease)	T1	T2	T3	T4
E. coli	33.51	40.93	61.13	78.26
Salmonella	50.48	57.74	75.68	75

Conclusion
Increased villus height or villus height to crypt depth ratio is a useful criterion to estimate and suggestive of greater nutrient absorption and this was observed in the present study in the pigs fed T3 and T4 because N-acetyl glucosamine could increase intestinal villus height. Thus, the increase of villus height after dietary supplementation with COS might lead to enhanced nutrient digestibility and absorption in weaned pigs. The results of study indicated that feeding of nanochitosan at 0.1% during pre-weaning phase and 0.5% during grower phase significantly improved the growth performance interms of weight gain and reduce gut pathogen and which could be used as an effective alternative to the use of antibiotics as growth promoters in swine production.

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