UTILIZATION OF SHRIMP SHELLS CONTAINING CHITOSAN AS A SOURCE OF PREBIOTIC IN CROSSBRED PIGS

By

M. YUGANDHAR KUMAR M.V.Sc.,
ID. No. TVD/2013-04

THESIS SUBMITTED TO

SRI VENKATESWARA VETERINARY UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY
(ANIMAL NUTRITION)
IN THE FACULTY OF VETERINARY SCIENCE

DEPARTMENT OF ANIMAL NUTRITION
COLLEGE OF VETERINARY SCIENCE
SRIVENKATESWARAVETERINARYUNIVERSITY
TIRUPATI- 517 502

2017

CERTIFICATE

Mr. M.YUGANDHAR KUMAR has satisfactorily prosecuted the course of research and that the thesis entitled "UTILIZATION OF SHRIMP SHELLS CONTAINING CHITOSAN AS A SOURCE OF PREBIOTIC IN CROSSBRED PIGS" submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis and part thereof has not been previously submitted by him for degree of any university.

Place: TIRUPATI
Date :

(Dr. J.V.RAMANA)
Major Advisor
Controller of Examinations
S.V.Veterinary University
Tirupati.

CERTIFICATE

This is to certify that the thesis entitled **"UTILIZATION OF SHRIMP SHELLS CONTAINING CHITOSAN AS A SOURCE OF PREBIOTIC IN CROSSBRED PIGS"** submitted in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in the faculty of Veterinary Science of **Sri Venkateswara Veterinary University, Tirupati,** is a record of the bonafide research work carried out by **Mr. M. Yugandhar Kumar** under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of the investigations have been duly acknowledged by the author of the thesis.

(Dr. J.V.RAMANA)
Chairman of the Advisory Committee

Thesis approved by the Student's Advisory Committee

Chairman	**:**	**Dr. J. V. Ramana** Controller of Examinations S.V. Veterinary University, Tirupati	----------
Member	**:**	**Dr. A. Ravi** Professor & Head Department of LFC College of Veterinary Science Tirupati	----------
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Member	**:**	**Dr. A. V. N. Sivakumar** Asst.Professor Department of Vety. Physiology College of Veterinary Science Tirupati	----------

DECLARATION

I, **M. Yugandhar Kumar** hereby declare that the thesis entitled **"UTILIZATION OF SHRIMP SHELLS CONTAINING CHITOSAN AS A SOURCE OF PREBIOTIC IN CROSSBRED PIGS"** submitted to Sri Venkateswara Veterinary University, Tirupati for the degree of **DOCTOR OF PHILOSOPHY** in the Faculty of Veterinary Science is the result of original work done by me. I also declare that the material contained in this thesis has not been published earlier.

Place : TIRUPATI **(M.YUGANDHAR KUMAR)**

Date :

ACKNOWLEDGEMENTS

I wish to express my deep sense of gratitude and profound thanks to my major advisor ***Dr. J. V. Ramana,*** *Controller of Examinations, S.V. Veterinary University, Tirupati* for *his valuable guidance during the investigation and in completion of my thesis.*

I am highly thankful to ***Dr.A.Ravi,*** *Professor and Head, Department of LFC,College of veterinary science, Tirupati, member of my advisory committee, for his helping hand and timely suggestions during my experimental trial and writing of my thesis..*

I express my deep sense of gratitude to ***Dr J.Suresh,*** *Professor and Head, Department of LPM, College of Veterinary Science, Tirupati and* ***Dr. A. V. N. Sivakumar,*** *Asst. Professor, Dept. of Vet. Physiology, Members of my Advisory Committee, for their constant guidance and support throughout execution of my research work.*

I express my special thanks to ***Dr. T. S. Chandrasekar Rao****, Dean of veterinary science, SVVU, Tirupati, for his administrative and moral help extended during my research work. I express my sincere thanks to* ***Dr. K. Sakunthala Devi,*** *Sr. Scientist and Head, AICRP on pigs, Tirupati, for permitting me to carry out my research work at AICRP on pigs, Tirupati, and I also express my thanks to* ***Dr. P. Eswara Prasad****, Associate Dean, CVSC., Tirupati for extending his support during my research work.*

I am also thankful to ***Dr. Devasena****, Professor, Department of LFC, College of Veterinary Science, Tirupati,* ***Dr. P. Kavitha,*** *Assistant Professor, Department of LFC, for their kind help during period of my study. I acknowledge with sincerity the guidance and help rendered to me by* ***Dr. Laxman****, Professor, RUSKA Labs and* ***Dr. Kranthi,*** *Asso. Professor, Dept. of Anatomy, NTR College of Veterinary Science, Gannavaram during micrometry study. I am very affectionate to thank my colleagues in PhD,* ***Dr. Lavanya*** *and* ***Dr. Subbarayudu, Dr. Chandrasekhar Reddy, Dr. Kishore,*** *M.V.Sc. students for their cooperation during my research work.*

*It gives me immense pleasure to express my love and deep sense of honour to my **parents** for their moral support, love and motivation throughout my life and it is my duty to express my heartfelt thanks to my beloved wife **Madhavi** for her patience, love, moral support and source of personal care and inspiration during period of study. I remember the relaxation given to my mind during thesis writing by my beloved daughter, **Dr. Harika** by her lovely smile.*

*I am deeply delighted at this moment to extend my thanks to the workers of AICRP on Pigs College of Veterinary Science, Tirupati, I also feel delighted to express my thanks to **Sri. V. ChandraSekhar,** Jr. Asst. for support during my thesis writing.*

*On top priority I strongly feel the support of the **Lord of Seven Hills** which made me to move in the right direction uninterrupted and it was proved again that belief in **GOD** never fails.*

(M.YUGANDHAR KUMAR)

LIST OF ABBREVIATIONS

%	:	Per cent
>	:	Greater than
<	:	Less than
≥	:	Greater than or equal to
≤	:	Lesser than or equal to
AOAC	:	Association of Official Analytical Chemists
Avg	:	Average
ADF	:	Acid Detergent Fibre
AGP	:	Antibiotic growth promoters
ATTD	:	Apparent Total Tract Digestibility
ADFI	:	Average daily feed intake
ADG	:	Average daily gain
AICRP	:	All India Co-ordinated Research Project
BGA	:	Brilliant green agar
BW	:	Body weight
Ca	:	Calcium
cm	:	centimeter
CF	:	Crude fibre
CP	:	Crude protein
CFU	:	Colony forming units
DE	:	Digestible energy
DM	:	Dry matter
DORB	:	Deoiled rice bran
FM	:	Faecal matter

EE	:	Ether extract
EFU	:	Efficiency of Feed Utilization
EMB Agar	:	Eosin Methylene blue
FA	:	Fatty acids
FCR	:	Feed conversion ratio
FTIR	:	Fourier transform infrared spectroscopy
Fig	:	Figure
GE	:	Gross Energy
G: F	:	Gain: Feed
g	:	gram
g/d	:	grams per day
HC	:	Hemicellulose
HDL	:	High Density Lipoprotein
kg	:	kilogram
Kcals	:	Kilo calories
LDL	:	Low Density Lipoprotein
ME	:	Metabolisable energy
mm	:	millimetre
ml	:	millilitre
mg/dl	:	milligram/deciliter
N	:	Nitrogen
NDF	:	Neutral Detergent Fibre
NE	:	Net Energy
NFE	:	Nitrogen-Free Extract
ND	:	Not detectable

NS	:	Not significant
NC	:	Negative Control
NRC	:	National Research Council
OM	:	Organic Matter
P	:	Phosphorus
PC	:	Positive Control
Psi	:	Pascal
RBC	:	Red blood cells
SBM	:	Soybean meal
SSM	:	Shrimp shell meal
SSW	:	Shrimp shell waste
SD	:	Standard deviation
SE	:	Standard error
SEM	:	Scanning electron microscopy
TA	:	Total Ash
USDA	:	United states department of Agriculture
WBC	:	White blood cells

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Degree to which it is submitted	:	**DOCTOR OF PHILOSOPHY**
Discipline	:	**ANIMAL NUTRITION**
Major Advisor	:	**Dr. J. V. RAMANA** **CONTROLLER OF EXAMINATIONS,** **S.V.VETERINARY UNIVERSITY,** **TIRUPATI.**
University	:	**SRI VENKATESWARA VETERINARY UNIVERSITY, TIRUPATI.**
Year of submission	:	**2017**

ABSTRACT

Shrimp shell meal (SSM) containing chitosan was evaluated for its prebiotic effect during creep, grower and finisher phases of swine feeding. During creep phase a total of 240 pre-weaned piglets were assigned at random to 5 dietary treatments of control diet (T_1), T_1 supplemented with antibiotic (T_2), while in treatments T3, T4, and T5 dried and autoclaved SSM containing 15.5% chitosan was included at 0.5, 1.0 and 1.5%, respectively such that the rations contained 700, 1500 and 2300 mg chitosan/kg. Each treatment contained six replicate pens with eight piglets per pen. From 240 piglets at creep stage, 40 pigs were selected as and when they attained 15 kg body weight for growth studies during grower phase (15-35 kg) and were shifted to the corresponding diet during finisher phases (35-70 kg live weight). During these phases, the standard ration (T1) was supplemented with chlorotetracyclin (T2) while dried SSM was included at 2.5 (T3), 5.0 (T4) and 7.5% (T5), as a source of chitosan. The experimental diets were formulated as per NRC, 1998 and the diets were isocaloric and isonitrogenous.

The growth performance and nutrient digestibility were studied. Two pigs per treatment at the end of creep and grower phases and 6 pigs per treatment at the end of finisher phase were slaughtered to study the small intestine morphological structures, gut pathogen load, serum biochemical profile and haematological parameters while carcass characteristics were studied after finisher phase.

During creep phase, the initial litter weight (kg) was not significantly different among treatments whereas the final weight (kg) was higher ($P<0.01$) in piglets fed T2 toT5 than those fed on T1 and the values were 66.01 (T1), 73.33 (T2), 75.16 (T3), 75.83 (T4) and 79.56 (T5). There was no significant difference among treatments in total feed intake (kg). The feed per kg gain was higher ($P<0.05$) in T1 than in other treatments and the values were 0.88, 0.78, 0.73, 0.65 and 0.71 for T1 to T5 fed piglets, respectively. The height of villi in duodenum, jejunum and ileum as well as the ratio of villi height to crypt depth increased ($P<0.01$) with increasing levels of SSM. The height of villi (µm) in duodenum, jejunum and ileum of pigs fed on T4 and T5 ration was significantly higher ($P<0.01$) than in other treatments and the values were 166.8, 177.4, 217.8, 393.4 and 315.2; 171.0, 243.0, 245.0, 254.2 and 246.6; 174.4, 184.4, 177.6, 247.4 and 226.8 in duodenum, jejunum and ileum for T1 to T5 fed pigs, respectively. The ratio of villus height to crypt depth (VH/CD) which is a useful criterion for estimating the digestive capacity in the small intestine was highest ($P<0.01$) in pigs fed T4 ration when compared to other treatments and the values were 0.94, 1.26, 1.14, 2.08 and 1.18; 1.02, 1.27, 1.08, 2.08 and 1.12; 0.97, 1.08, 1.01, 1.76 and 1.06 in duodenum, jejunum and ileum for T1 to T5 fed pigs, respectively. There was a reduction in the *E.Coli* and *Salmonella* count in gut content of piglets fed diets containing antibiotic and shrimp waste when compared to control

group and the values (cfu/g) were 76.35, 24.09, 32.23, 23.67 and 19.37 (*E.coli*); 53.52, 31.13, 40.29, 29.98 and 16.99 (*Salmonella*) in pigs fed T1 to T5, respectively.

During grower phase, the ADG (g) was higher (P<0.01) in T4 (348) or T5 (324) than in T3 (310), T2 (303) and T1 (267) fed pigs. The ADFI (kg) was also higher (P<0.01) in T5 (0.80) or T4 (0.78) fed pigs than in T1 (0.73), T2 (0.75) and T3 (0.75) fed pigs which was not significantly different. The quantity (kg) of feed consumed per kg gain and the cost of feed per kg gain (Rs) were higher (P<0.01) in T1 fed pigs than in other treatments and the values were 2.77 and 61.24 (T1), 2.47, and 54.47 (T2), 2.42 and 53.46 (T3), 2.26 and 49.84 (T4) and 2.46 and 54.40 (T5), respectively.

During finisher phase, the initial, final and total weight gain (kg) were not significantly different among the treatments and the values were 35.50, 70.75 & 35.25; 35.08, 70.66 & 35.58; 35.91, 71.33 & 35.41; 35.75, 70.91 & 35.16 and 35.75, 71.41 & 35.66, respectively for T1 to T5 fed pigs. The number of days taken was lower (P<0.01) in T4 (72) or T5 (79) than in T2 (90), T3 (80) or T1 (104) fed pigs. The ADG (g) was higher (P<0.01) in T4 (485) followed by T5 (451) than in T1 (338), T2 (393) or T3 (439) fed pigs. The height of villi in duodenum, jejunum and ileum and the ratio of villi height to crypt depth increased (P<0.01) with increasing levels of SSM. The height of villi in duodenum, jejunum and ileum of pigs fed on T4 ration was significantly higher (P<0.01) than in other treatments and the values were 177.4, 190.6, 176.8, 202.8 and 186.2; 176.4, 182.8, 178.4, 187.2 and 188.4; 172.0, 182.0, 171.4, 204.0 and 180.8 (µm) in duodenum, jejunum and ileum for T1 to T5 fed pigs, respectively. There was a reduction in the *E. Coli* and *Salmonella* count in gut content of pigs fed diets containing

antibiotic and shrimp waste when compared to control group and the values (cfu/g) were 72.36,25.81, 35.70, 26.77 and 18.37 (*E.coli*); 61.25, 21.49, 34.61, 22.65 and 14.17 (*Salmonella*) in pigs fed T1 to T5, respectively.

The digestibility of major nutrients except ether extract was increased when pigs were offered diets supplemented with SSM during finisher phase. The digestibility of DM, CP, CF and NFE was significantly higher (P<0.01) in pigs fed T4 and T5 rations than in other treatments and the values were 85.27, 85.84, 84.08, 87.69 and 86.61; 85.00, 85.98, 85.13, 89.65 and 85.01; 53.79, 56.78, 56.80, 64.75 and 64.85; 90.37, 90.91, 90.76, 93.22 and 93.15 in pigs fed T1 to T5, respectively, However, EE digestibility was significantly higher (P<0.01) in T1 and T2 compared to T3, T4 and T5 fed pigs. The nitrogen intake (g/d) was higher (P<0.01) in pigs fed T1 than in other treatments and was lowest in pigs fed T4 and it might be due to differences in feed intake during metabolism trial.

The serum total protein concentration was increased (P<0.01) in response to SSM supplementation compared to control group which indicated that the protein status of the pigs had improved. The total protein (g/dl) content was highest (P<0.01) in T4 fed pigs than in T1, T2, T3 and T5 and the values were 5.41, 5.98, 5.96, 6.75 and 5.95, respectively for T1 to T5 pig feds. The albumin (g/dl) content was not significantly different among the treatments and the values were 4.25, 4.41, 4.43, 4.15 and 4.26, respectively for T1 to T5 fed pigs. The globulin (g/dl) and IgG (mg/dl) content was highest (P<0.01) in T4 fed pigs and was in order of T4>T5>T2>T3>T1 fed pigs and the values were 1.16, 649.16; 1.57, 695.16; 1.52, 694.16; 2.60, 716.50 and 1.69, 697.00, respectively for T1 to T5 fed pigs. The increased total protein concentration was primarily due to an improved globulin concentration, since there was increase in the serum IgG concentration.

During creep phase, there was a decrease in the serum triglycerides, total cholesterol, LDL cholesterol and increase in HDL cholesterol and the values were 76.25, 70.75, 69.00, 64.25 and 65.25 (total cholesterol); 66.80, 65.25, 66.00, 58.50 and 61.75 (triglycerides); 41.00, 43.00, 42.25, 45.00 and 41.75 (HDL); 53.25, 27.75, 26.75, 19.25 and 23.50 (LDL) **(**mg/dl), respectively for T1 to T5 fed pigs. During grower phase also, there was a decrease in the total cholesterol, triglyceride and LDL cholesterol and increase in HDL cholesterol in pigs fed T2 to T5 than in T1 and the values were 76.75, 72.00, 71.75, 64.75 and 69.00 (total cholesterol); 72.25, 68.00, 68.25, 60.25, 68.00 (triglycerides); 41.50, 43.25, 43.75, 44.25 and 44.25 (HDL); 35.25, 28.75, 28.00, 20.50 and 24.75 (LDL) and the same trend was observed even in finisher phase and the values were 77.33, 75.33, 74.08, 67.40 and 71.08 (total cholesterol); 72.80, 70.00, 68.58, 61.50 and 65.83 (triglycerides); 43.08, 45.83, 46.16, 48.75 and 46.66 (HDL); 34.25, 29.50, 27.91, 18.65 and 24.41 **(**mg/dl**)** (LDL).

The major finding emerging from the current study was that indicators of gut function such as nutrient digestibility, small intestine morphology and ratio of villus height to crypt depth were augmented with supplementation of SSM, resulting in higher body weight gain and superior feed/kg gain ratio and it was concluded that SSM at 1% during creep stage and at 5% during grower and finisher phases was optimum as a source of chitosan to replace antibiotic feed additive. Further, the enhanced small intestinal morphology observed during creep phase was beneficial in sustaining the same trend of improved gut function, nutrient utilization and better growth performance during grower and finisher phases where SSM was included at higher levels than during creep phase.

CHAPTER – I

INTRODUCTION

Demand for animal protein for human consumption is rising globally at an unprecedented pace. Use of antibiotics as growth promoters or to control diseases (Yang *et al.* 2015; Wan *et al*. 2017) in swine production contribute to the spread of drug-resistant pathogens in both livestock and human, posing significant public health threat (Gois *et al.* 2016). However, these negative effects of antibiotics have become increasingly prominent and consumers are concerned about antibiotic residues in meat products. Moreover with the ban of antibiotic growth promoters by several countries (Simon *et al.* 2003), extensive research work has become a continuous process leading to identification of many potential alternative growth promoters such as acidifiers, probiotics, prebiotics etc., to reduce or eliminate the use of antibiotics in animal feeds.

In recent years, multiple reports have described the beneficial effects of dietary oligosaccharides such as chito-oligosaccharides by enhancing host health status, modulating the intestinal microflora (Han *et al.* 2007; Liu *et al.* 2008; Wang *et al.* 2009; Yang *et al*. 2012; Xu *et al*. 2014), improving the small intestine morphological structure (Xu *et al.* 2013; Swiatkiewicz *et al.* 2015; Wan *et al.* 2017) and increasing the digestibility of nutrients (Suthongsa *et al.* 2017; Dias *et al.* 2017). Furthermore, dietary chito-oligosaccharides serve not only as growth promoters for beneficiary bacteria but also effectively inhibit the growth and activity of pathogenic microorganisms (Rhoades *et al.* 2006; Han *et al.* 2007; Xu *et al.* 2012).

Chitosan obtained from chito-oligosacharides (COS) is the second most abundant carbohydrate polymer in nature (Ameh *et al.* 2015; Krishnaveni and Ragunathan, 2015; Daniel Elich Ali Komi and Michael R Hamblin, 2016; Wan *et al.* 2017) after cellulose and is widely distributed in exoskeleton of shrimp and crab (Lodhi *et al.* 2014; Arafat *et al*, 2015; Azuma *et al.* 2015; Yue *et al.* 2017). It is nontoxic, biodegradable carbohydrate polymer (Goiri *et al.* 2010), containing amino and hydroxyl groups (Liao *et al.* 2007; Ramya *et al.* 2012; Arafat *et al.* 2015), which give chitosan many biological activities such as antimicrobial, (Limam *et al.* 2011; Benhabiles *et al.* 2012; Azuma *et al.* 2015 and Dias *et al.* 2017), fungistatic and hemostatic (Dutta *et al.* 2004; Hui *et al.* 2012), hypocholesterolemic (Kerch. 2015), antioxidant (Trung and Bao, 2015; Xie *et al.* 2016; Xiong *et al.* 2015), anti inflammatory (Huang *et al.* 2016), antitumor (Yogeshkumar *et al.* 2013; Azuma *et al.* 2015) and immune-stimulatory effect (Yin *et al.* 2008; Ignacak *et al.* 2010; Wum *et al.* 2015). It also binds to mammalian and microbial cells aggressively (Dutta *et al.* 2004) when fed as dietary additive to farm animals. These properties of chitosan, unlike many other kinds of feed additives, were often reflected in improved growth performance of animals (Yang *et al.* 2012; Yuan and Chen, 2012; Xu *et al.* 2014; Swiatkiewicz *et al.* 2015; Wan *et al.* 2017). Notably, COS has been used as feed supplement to improve growth performance and nutrient digestability in pigs (Wan *et al.* 2017). Shrimp waste meal containing chotosan as feed supplement is a part of waste management in the shrimp processing industry. Every year 6 to 8 million tonnes of crab, shrimp and lobster shells are produced globally (Yan and Chen, 2015). In India the shrimp processing industry is one of the major food processing industries and significant amount of waste (shrimp heads, exoskeleton, and soluble components) estimated to be around 1.25 to 1.50 thousand tons per annum is

available (Ramyadevi *et al.* 2012). Chitin, the solid fraction available from this waste is estimated as 60 to 80 thousand tons per annum (Kumari *et al.* 2016).

In view of scant reports on utilization of shrimp shells in swine diets in India, the present study was taken up with the following objectives.

1) To study the chemical composition of the shrimp shells
2) To study the effect of shrimp shell inclusion on growth performance of crossbred pigs during pre weaning, grower and finisher stages
3) To study the effect of shrimp shell inclusion on nutrient utilization, gut pathogen population, small intestine morphology and haematological characteristics of pigs and
4) To study the effect of shrimp shell inclusion on carcass characteristics, lipid profile and economics of pork production.

CHAPTER – II

REVIEW OF LITERATURE

Antibiotics have played a major role in the growth and development of swine industry for more than 50 years. Their efficiency in increasing growth rate, improving feed utilization and reducing mortality from clinical disease is well documented. However, consumers are becoming increasingly concerned about drug residues in meat products (Vondruskova *et al.* 2010). In addition, it has been suggested that the continuous use of antibiotics may contribute to a reservoir of drug resistant bacteria which may be capable of transferring their resistance to pathogenic bacteria in both animals and human (Van der Fels-Klerx *et al.* 2011). As a result, many countries have banned or are banning theinclusion of antibiotics in swine diets as growth promoters. In the past two decades, intensive research have been focused on the development of alternativesto antibiotics such as probiotics, prebiotics, acidifiers etc., to maintain swine health and performance. In recent years, multiple reports have described the beneficial effects of dietary oligosaccharides such as chito-oligosacharides which are widely distributed in exoskeleton of shrimp shells (Wan *et al.* 2017).

Shrimp shell meal, a by-product of shrimp processing industry, is the undecomposed ground dried waste and is one of the important sources of chitosan. It is produced by sun-drying and the major components of shrimp shells are protein, chitin, minerals and carotenoids. Many studies have reported about utilization of shrimp shell meal containing chitosan as an alternative to antimicrobials. Most of the studies carried out were of short duration and emphasizing on the prebiotic nature of COS of shrimp shell, particularly in preweaned pigs.

In view of very limited reports on utilization of shrimp shell waste during creep, grower and finisher stages of swine, the present study was an attempt to elucidate the prebiotic effect of feeding shrimp shell waste containing chitosan on growth promotion, nutrient digestibility, intestinal microflora, lipid profile, haematological characteristics, intestinal morphology and carcass characteristics in pigs. The research work carried by various workers on utilization of shrimp shell waste as a source of prebiotic and its effect on performance of pigs is presented in this Chapter.

2.1 Prebiotics in pig nutrition

Prebiotics are nondigestible feed ingredients that can positively affect the animal organism by stimulating the activity and growth of beneficial native bacteria in the gastrointestinal tract and eliminate the pathogenic ones. The commonly used prebiotics are mannan-oligosaccharides, fructo-oligosaccharides (FOS), oligofructose, inulin trans-galacto-oligosaccharides (TOS), chito-oligosaccharides(COS), gluco-oligosaccharides, glyco-oligosaccharides, lactulose, lactitol, malto-oligosaccharides, xylo-oligosaccharides, stachyose and raffinose etc. (Davis *et al.* 2004;Grela*et al.*2006; Miguel *et al.*2006; Jacela *et al.* 2010; Limam*et al.* 2011; Zhao *et al.* 2012; Halas and Nochta,2012; Thacker, 2013; Xu *et al.* 2014; Swiatkiewicz *et al.* 2015; Wan *et al.* 2016; Dias *et al.*2017).

2.2 Chitosan as a prebiotic

Chitosan the second most abundant carbohydrate polymer in nature after cellulose and widely distributed in exoskeleton of shrimp shells and crab (Li *et al.* 2009; Lodhi *et al.* 2014; Wan *et al.* 2017) is nontoxic, biodegradable carbohydrate polymer (Goiri *et al.* 2010) that contains amino and hydroxyl groups (Liao *et al.* 2007), which give chitosan many biological activities such as antimicrobial

activity (Han *et al.* 2007; Limam *et al.* 2011; Benhabiles *et al.* 2012) when fed as dietary additive for farm animals. Due to these properties, chitosan can be used as a pro-growth dietary supplement for animals, as well as an antibiotic alternative (Yang *et al.* 2012; Yuan and Chen, 2012; Xu *et al.* 2014; Swiatkiewicz *et al.* 2015; Xiong *et al.* 2015; Suthongsa *et al.* 2017; Wan *et al.* 2017).

2.3Extraction of chitosan

The main commercial process for chitosan extraction from shrimp shell waste is based on demineralization by acid treatment, deproteinization by alkali treatment and deacetylation again by strong alkali.

Islam *et al.* (2011) reported 15.21% chitosan yield from shrimp processing waste. Puvvada *et al.* (2012) reported 34% chitosan yield from crude chitin collected from the exoskeleton of shrimp by chemical method. Selimot *et al.* (2013) also reported 14.7% chitosan yield from raw shrimp shells.

Kamala *et al.* (2013) observed that the yield of chitin was 32% of total weight of the dried shrimp shells and the yield of chitosan was 54.31% from chitin. The infrared spectroscopy of the structural changes in initial chitin and chitosan as confirmed by FTIR spectroscopy with standard chitin and chitosan. Ameh *et al.*(2015) reported 15-20% yield of chitosan from shrimps by acid demineralization. Arafat *et al.*(2015) observed the yield of 19.0% chitosan from shrimp shell waste. Yan and Chen (2015) reported yield of 15-40% chitin from shells whereas Jose Carlos *et al.*(2016) observed 33% yield of chitin.

2.4 Characterization of chitosan

Chitosan which was obtained by deacetylation of chitin can be characterized by using FTIR spectroscopy.

Puvvada *et al.* (2012) reported the bands of spectrum in standard chitosan cm^{-1} as 3858 for OH stretching, 3609 for NH stretching, 1643 for amide bond, 1552 for amide 2 bond, 1374 for amide 3 bond, CH^2 bending and CH^3 deformation at 1421, asymmetric bridge O2 stretching at 1313, CO-stretching at 1022 and NH out of plane bending at 564.

Ramya *et al.* (2012) reported the absorption band at 3434 cm^{-1} corresponds to –OH and NH stretching frequency. The band at 1598 cm^{-1} corresponds to C=O stretching vibration and 1426cm^{-1} corresponds to C-H and O-H deformation vibrations. The peak 777 cm^{-1} band showed CH^2 rocking and the peak 680 cm^{-1} corresponds to N-H bending. Kamala *et al.* (2013) reported the bands of spectrums in FTIR spectra as 1568 for amide 2 bond, 929 for amide 3 bond.

Selimot *et al.* (2013) revealed the main absorbance at 3439 cm^{-1} (OH stretching) at 1654cm^{-1} (-C=O stretching of amide bonds) or stretching and vibrating of (-C=O) secondary amide at 1558cm^{-1} (N-H) bending of amide bonds. Mahalakshmi Devi *et al.* (2014) observed prominent peaks 2926 cm^{-1},1746 cm-^{1}, 1565 cm^{-1} and 1465 cm^{-1}in C-H stretching, C=O stretching, C=N stretching and OH bending, respectively.

Arafat *et al.* (2015) reported strong absorption bond at 3454 cm^{-1} due to OH and amine NH symmetrical stretching vibrations. A peak at 2926 cm^{-1} was due to symmetric CH2 stretching vibration. The sharp peak at 1384 cm^{-1} was reported and carbonyl at 1629cm^{-1} ensured the FTIR spectra of chitosan.

Absorbance bands were observed by Ameh *et al.* (2015) at 3268, 1643, 1552, 1421, 1822, 893 and 752cm^{-1} which confirms the structure of chitosan.

Kumari *et al.* (2016) reported that formation of two separate bands in the region of 1662-1630cm^{-1}confirmed the presence of a chitin in shrimp. In the FTRI spectra the peak noticed at 1555cm^{-1}corresponds to N-H bending of the secondary amide II band of –CONH-. Further bands are observed in the region of 1380-1460cm^{-1} and are attributed to the symmetric and asymmetric bending vibrations of the methyl groups. Li *et al.* (1998) also found that the peak at 1415cm^{-1} indicating C-H bending vibrations of $–CH^2$.

2.5 Availability of shrimp shell waste

The shrimp processing industry has been rapidly growing in India. Gopakumar (2002) and Mathew and Nair (2006) have reported that the availability of shrimp processing waste in India is 1,00,000 tons per annum.

Ramyadevi *et al.* (2012) reported that the processing waste generated in Indian shrimp processing industries would be around 1,25,000 to 1,50,000 tonnes per annum. Gortari *et al.* (2013) reported that the annual world wide production of chitin is approximately 10^{10} to 10^{12} ton per annum.

Yan and Chen (2015) reported that every year, 6 to 8 million tonnes of crab, shrimp and lobster shells are produced globally and these dried shrimp shells are valued at $ 100-200 per tonne. Panchakshari *et al.,* (2016) reported that 60,000 to 80,000 tonnes of chitinous wastes are produced annually in India and at present only a small quantity of shell waste is utilized for animal feed or chitin extraction.

2.6 Chemical composition of shrimp shell waste

The chemical composition of shrimp shell waste vary significantly depending upon the nature of the processing (Table1). The most common methods of processing are hand-deheading and mechanical-peeling. The waste produced from each process varies in the level of chitin, protein, non-protein nitrogen and calcium carbonate.

Meyers (1986) reported 51.7% CP and 9.0% chitin in sun-dried shrimp meal. Balogum and Samsons (1992) have reported 28.43, 34.00, 1.11 and 0.06 per cent of DM, CP, Fat and CF, respectivelyon DM basis in shrimp waste. Sabry (1992) reported moisture content of 20.0% in shrimp-shell waste and 6.0, 21.4, 27.9 and 40.0 per cent of ash, chitin, crude protein and calcium carbonate, respectivelyon DM basis.

Rosenfeld *et al.* (1997) reported 50.89, 6.31, 15.64, 8.92, 5.21 and 1.47 per cent of CP, EE, total ash, CF, calcium and phosphorus, respectively on DM basis. Fanimo and Oduguva (1999) reported 46.3, 9.04, 17.04, 4.3, 7.0, 3.03 and 9.82 percent and 2500 ME (kcal/kg) of CP, EE, TA, CF, Ca, P, Chitin on DM basis, respectively. Ngoan *et al.* (2000) reported 44.0, 7.3, 18.1, 22.8, 10.5 and 1.2 % CP, EE, Chitin, Ash, Calcium and Phosphorus (on DM basis), respectively. Gernat (2001) reported 52.70, 6.20, 20.41, 11.38, 5.21 and 1.47 per cent CP, EE, Total ash, CF, Calcium and available Phosphorus, respectively (as-fed basis). Gopakumar (2002) observed 35-40, 3-5, 30-35 and 15-20 per cent of CP, EE, TA and Chitin, respectively.

Fanimo *et al.* (2004) collected shrimp waste from the shrimp processing plant, sun dried immediately and analysed for chemical composition and observed 39.45, 12.30, 9.00, 24.00, 15.77 and 0.45 per cent CP, CF, EE, ash, calcium and

phosphorus, respectively on DM basis. Oduguwa *et al.* (2004) analysed proximate composition and reported 40.2, 4.8, 10.9 and 16.2 per cent CP, EE, CF and ash, respectively on DM basis.

Okoye *et al.* (2005) reported 46.3,4.3,9.04,17.04,9.82,7.00, 3.03 and 2500 CP, CF, EE, Ash, Chitin, Calcium, Phosphorous % and ME(Kcal/kgDM), respectively on DM basis. Khempaka *et al.* (2006) reported 39.32, 0.94, 26.73, 29.75, 6.05, 0.97, 30.44 % and 1350 CP, EE, TA, CF, Ca, P, Chitin % and ME (Kcal/kg), respectively. Munguti *et al.* (2006) reported 63.5, 1.3, 5.0 and 22.8% CP, EE, CF and Ash (on DM basis), respectively. Holanda and Netto (2006) reported 9.30, 54.40, 21.20 and 11.9 per cent chitin, CP, Ash and EE, respectively on DM basis.

Ingweye *et al.* (2008) reported 48.3, 6.3, 17.55, and 13.3 per cent of CP, EE, TA and CF (on DM basis), respectively. Mahata *et al.* (2008) analysed for proximate composition and reported 24.03, 26.89, 5.14, 25.60 and 10.47 per cent CP, CF, EE, Total ash and NFE, respectively (DM basis). Nguyen (2009) reported 10-20% calcium, 30-65% protein and 8-10% chitin on dry basis in shrimp waste. Ravichandran *et al.* (2009) have reported 32.5, 1.5, 9.8, 8.7, 12.3 and 26.6 CP, crude carbohydrate, crude lipid, CF, moisture and total ash, respectively (% dry weight). Aktar *et al.* (2011) reported 48.3, 5.75, 12.9, 3.5, 1.0 and 1870 CP, EE,CF, Ca, P and ME(Kcal/kg), respectively (% dry weight).Camargo *et al.*,(2011) reported 49.0, 27.0, 18.2 and 4.83 per cent CP, total ash, CF and EE, respectively on DM basis. Ehigiator *et al.* (2011) reported 5.97, 34.67, 38.50, 4.67, 0.03 and 21.83 per cent moisture, ash, CP, EE, CF and NFE, respectively. Khempaka *et al.* (2011) reported 36.69, 10.28, 21.77, 19.49, 4.92, 1.2, 18.99 and 1515 CP, EE, TA, CF, Ca, P, Chitin % and ME(Kcal/kg), respectively.

The chemical composition of redspotted shrimp waste was investigated by Sanchez-Camargo *et al.* (2011) and reported 49.0, 27.0, 18.2, 4.9, 4.83 total protein, ash, crude fiber, total lipids and ether extract on dry weight basis. Bellaaj *et al.* (2012) reported moisture content of 53.7 percent and the protein, ash, fat and chitin contents of the material was 24.9, 46.1, 6.2 and 25.2%, respectively, of dry weight.

Ehigiator and Oteria (2012) reported 68.77, 6.87, 10.55, 0.40, 10.25 and 0.58 per cent protein, fat, ash, fibre, moisture and carbohydrates, respectively. Okonkwo *et al.* (2012) reported 20.0, 7.44, 24.5 and 8.46 per cent of CP, EE, TA and CF, respectively.The crude chitin was collected from exoskeleton of shrimp by Puvvada *et al.* (2012) processed to obtain chitosan and reported 35.49% yield of chitosan from chitin. Kamala *et al.* (2013) reported 32% yield of chitin in the total weight of dried shrimp shells 54.31% of chitosan after N-acetylation of chitin. Puga-lopez *et al.* (2013) reported 73.91 per cent moisture, 20.04, 2.26 and 1.27 per cent protein, ash and crude lipid, respectively.

Selimot *et al.* (2013) reported 32.61, 24.95, 1.65, 0.3 and 14.70 per cent CP, CF, Fat, total ash and chitosan, respectively on DM basis. Yan and Chen (2015) reported 20-40% protein, 20-50% calcium carbonate and 15-40% chitin. Yugandharkumar and Sakunthala Devi (2015) reported 39.5, 4.8, 24.8 and 8.7 per cent CP, EE, TA and CF, respectively on DM basis in shrimp waste. Panchakshari *et al.* (2016) observed chitosan yield of 34% from Tiger shrimp and Jose Carlos Vilar Junior *et al.* (2016) observed extraction yields for chitin of 33% and for chitosan of 49%.

Table 1: Chemical composition and chitin (%) of shrimp shell meal (as reported by several authors)

CP	EE	TA	CF	Ca	P	Chitin	Reference
30-40	NA	NA	NA	30-50	NA	20-30	Nouri *et al.,* 2015
20-40	NA	NA	NA	20-50	NA	15-40	Yan and Chen, 2015
39.5	4.8	24.8	8.7	NA	NA	NA	Kumar and Sakunthala, 2015
32.6	1.7	0.3	24.9	NA	NA	14.7	Selimot *et al.,* 2013
20.1	1.3	2.3	NA	NA	NA	NA	Puga-lopez *et al.,* 2013
20.0	7.44	24.5	8.5	NA	NA	NA	Okonkwo *et al.,* 2012
68.7	6.8	10.5	0.4	NA	NA	NA	Ehigiator and Oteria, 2012
24.9	6.2	46.1	NA	NA	NA	25.2	Bellaaj *et al.,* 2012
38.5	4.7	34.6	0.1	NA	NA	NA	Ehigiator *et al.,* 2011
49.0	4.8	27.0	18.2	NA	NA	NA	Sanchez-Camargo *et al.* 2011
36.6	10.2	21.7	19.5	4.9	1.2	18.9	Khempaka *et al.,* 2011
49.0	4.8	27.0	18.2	NA	NA	NA	Camargo *et al.,* 2011
48.3	5.75	NA	12.9	3.5	1.0	NA	Aktar *et al.,* 2011
32.5	1.5	26.6	8.7	NA	NA	NA	Ravichandran *et al.,* 2009
30.0	NA	NA	NA	10.0	NA	8.0	Nguyen, 2009
24.03	5.14	25.6	26.89	NA	NA	NA	Mahata *et al.,* 2008
48.3	6.3	17.5	13.3	NA	NA	NA	Ingweye *et al.,* 2008
63.5	1.3	22.8	5.0	NA	NA	NA	Munguti *et al.,* 2006
39.32	0.94	26.7	29.75	6.05	0.97	30.44	Khempaka *et al.,* 2006
46.3	9.04	17.0	4.3	7.0	3.03	9.82	Okoye *et al.,* 2005
40.2	4.8	16.2	10.9	NA	NA	NA	Oduguwa *et al.,* 2004
39.5	9.0	24.0	12.3	15.77	0.45	NA	Fanimo *et al.,* 2004
35.0	3.0	30.0	NA	NA	NA	15.0	Gopakumar, 2002
55.7	6.2	20.4	11.38	5.21	1.47	NA	Gernat, 2001
44.0	7.3	22.8	NA	10.5	1.2	18.1	Ngoan *et al.,* 2000
46.3	9.04	17.0	4.3	7.0	3.03	9.82	Fanimo and Oduguva, 1999
50.89	6.31	15.6	8.92	5.21	1.47	NA	Rosenfeld *et al.,* 1997
27.9	NA	6.0	NA	NA	NA	21.4	Sabry, 1992
34.0	1.11	NA	0.06	NA	NA	NA	Balogum and Samsons, 1992
40.6	**5.2**	**20.9**	**11.9**	**7.5**	**1.5**	**16.7**	**Mean**

NA: Not available

2.7 Effect of dietary chitosan supplementation on small intestinal morphology

The small intestine is the main place for digestion and absorption of nutrients, and the intestinal mucosa plays an important role in these processes. Weanling stress can result in relatively quick changes in the intestinal mucosa morphology, which lead to a reduction in villus height and an increase in crypt depth (Xiao *et al.* 2013; Swiatkiewicz *et al.* 2015; Xiong *et al.* 2015; Suthongsa *et al.* 2017). Shortening of the villus decreases the surface area for nutrient absorption, which lead to poor nutrient absorption and reduced performance (Pluske *et al.* 1995). The crypt is the area where stem cells divide to permit the renewal of the villus, and a large crypt indicates fast tissue turnover and a high demand for new tissue. The ratio of villus height to crypt depth is a useful criterion for estimating the digestive capacity in the small intestine (Montagne *et al.* 2003). Masri *et al.* (2015) reported that villus crypt ratio is a single measure for evaluation of small intestine maturity and health in swine rather than villus height or crypt depth. Several authors have reported that chitosan of shrimp waste had beneficial effect on intestinal morphology.

Liu *et al.* (2008) performed a study aimed at evaluating the effects of diet supplementation with COS and reported that enhanced growth performance in weaning pigs was due to improvement of small intestine morphology. A total of 50 weaning pigs were selected and assigned randomly to 5 treatments including control, 3 diets with COS supplementation (100, 200 and 400 mg/kg) and another diet with chlortetracycline (CTC) (80 mg/kg) and reported that supplementation of COS at 100 and 200 mg/kg and supplementation of CTC improved ($P<0.05$) growth performance by increasing the villus height and villus: crypt ratio in the jejunum of weanling pigs and concluded that COS can be an effective alternative

to the use of antibiotic growth promoters to increase growth through enhancing small intestinal structure.

The results of earlier experiments (Khambulai *et al.* 2009) indicated that the improvement of production characteristics by dietary chitosan can probably be attributed to its positive effect on intestinal morphology, i.e., the presence of hypertrophied villi and epithelial cells in birds fed a diet supplemented with chitosan. Yang *et al.* (2012) studied to evaluate the effect of COS (100, 400 and 600 mg/kg) on the growth performance and intestinal morphology of weaned pigs and reported that supplementation of COS did not significantly affect villus height and crypt depth in the duodenum, jejunum and ileum

A study was conducted by Xiao *et al.* (2013) to investigate the mechanism of COS on growth performance. Three groups of piglets in individual pens were fed a corn-soybean meal diet containing no addition, 50 mg/kg chlortetracycline and 300 mg/kg COS for 21 days. Jejunal morphology and histology were analyzed under light microscope and observed increase ($P<0.05$) in villus length and villus height, but villus width and crypt depth were decreased ($P<0.05$) both in COS and chlortetracycline groups. He observed that chitosan and the antibiotics have similar effects in promoting piglet growth and concluded chitosan can replace chlortetracycline as a feed additive for piglets.

Xu *et al.* (2013) conducted an experiment to determine the effects of chitosan on the small intestinal morphology and concentrations of GH and IGF-I in serum of piglets, in order to evaluate the regulating action of chitosan on weaned pig growth through endocrine and intestinal morphological approaches. A total of 180 weaned pigs (35 d of age; 11.56±1.61 kg of body weight) were

selected and assigned randomly to 5 dietary treatments, including 1 basal diet (control) and 4 diets with chitosan supplementation (100, 500, 1,000 and 2,000 mg/kg, respectively). Each treatment contained six replicate pens with six pigs per pen. The experiment lasted for 28 d. The results showed that the average body weight gain (BWG) of pigs was improved quadratically by dietary chitosan during the former 14 d and the later 14 d after weaning ($p<0.05$).

Diets supplemented with increasing levels of chitosan increased quadratically the villus height of jejunum and ileum on d 14 ($p = 0.089$, $p<0.01$) and on 28 d ($p = 0.074$, $p<0.01$), meanwhile, chitosan increased quadratically the ratio of villus height to crypt depth in duodenum, jejunum and ileum on d 14 ($p<0.05$, $p=0.055$, $p<0.01$) and 28 ($p<0.01$, $p<0.01$, $p<0.01$), however, it decreased quadratically crypt depth in ileum on d 14 ($p<0.05$) and that in duodenum, jejunum and ileum on d 28 ($p<0.01$, $p<0.05$, $p<0.05$). Chitosan could quadratically improve growth in weaned pigs, and the underlying mechanism may due to improvement of the small intestines morphology and increase the serum GH concentration.

Swiatkiewicz *et al.* (2015) conducted experiments to determine the effects of chitosan on small intestinal morphology of piglets, in order to evaluate the regulating action of chitosan on weaned pig growth through endocrine and intestinal morphological approaches and reported chitosan increased quadratically the ratio of villus height to crypt depth in duodenum, jejunum and ileum on day 14 ($p<0.05$, $p=0.055$, $p<0.01$) and 25 ($p<0.01$, $p<0.01$, $p<0.01$), however, it decreased quadratically crypt depth in ileum on day 14 ($p<0.05$) and that in duodenum, jejunum and ileum on day 28 ($p<0.01$, $p<0.05$, $p<0.05$). They concluded that chitosan could quadratically improve growth in weaned pigs, and the underlying

mechanism may due to the increase of the serum GH concentration and improvement of the small intestines morphology.

Suthongsa *et al.* (2017) conducted experiments to study the effects of dietary levels of chito-oligosaccharide on ileal digestibility of nutrients, small intestinal morphology and crypt cell proliferation in weaned pigs and observed increased ($P<0.001$, $P<0.05$) villus height and the villus height/crypt depth ratio in duodenum, jejunum and ileum on day 28 and day 56. Wan *et al.* (2017) reported COS supplementation promoted an increase ($P<0.05$) in the duodenal villus height by 4.65%, compared to the control group.

2.8 Effect of dietary chitosan supplementation on immune response andantibacterial effects / gut pathogens

The different mechanisms of the antimicrobial activity in the gut of weaned pigs were evaluated by Han *et al.* (2007) and observed inhibited growth of harmful bacteria, measured as faecal*E. coli* and *Clostridium spp.* counts, in young pigs (25 kg body weight) fed diet supplemented with COS at 0.30 and 0.40% levels. Total anaerobic bacterial number increased from caecum to recturn in all treatments. The weekly total bacteria count was higher ($p<0.05$) in faeces of pigs fed COS than in untreated pigs at the 8^{th} week. The number of faecal *E. coli* in untreated pigs at 4^{th} wk was 7.35 log CFU/g compared to 6.71 and 6.54 log CFU/g in 0.1 and 0.3% COS-treated pigs, respectively. Similarly, at 8^{th} wk, faecal*clostridium spp.* were lower in pigs fed 0.3% COS (5.43 log CFU/g) than in untreated pigs (6.26 log CFU/g) and concluded that chito-oligosaccharide could improve feed efficiency in young pigs and inhibited the growth of harmful bacteria.

The study by Huang *et al.* (2007) was to evaluate the effect of different dietary levels of chitosan (0.005, 0.010, and 0.015%) on immune function in broilers. Compared with broilers fed a diet containing antibiotic (flavomycin), dietary supplementation with COS increased serum concentrations of IgG, IgA, and IgM, as well as enhancing immune organ development, with the greatest response at 0.10% COS addition. The authors emphasised that their results demonstrate the potential of COS to improve immune response in birds, and thus it can be used as an effective, antibiotic-like growth promoter for poultry.

Liu *et al.* (2008) studied the effect of dietary COS on the faecal microflora of weaned pigs. They found that dietary supplementation with COS (100, 200 and 400 mg/kg) reduced the incidence of diarrhoea and *E. coli* counts, whereas increased *Lactobacillus* counts in the faeces. *Lactobacillus* and *E. coli* in the faeces of each treatment were determined at 4 different time points (day 0, 7, 14 and 21) during the study. *Lactobacillus* counts on day 14 and 21 indicated that pigs fed diets supplemented with COS at 100, 200 and 400 mg/kg had greater ($P<0.05$) *Lactobacillus* counts than did pigs in the negative control and CTC treatments. In addition, dietary supplementation of CTC decreased ($P<0.05$) the counts of *E. coli* shed in the faeces on day 21 compared with the negative control diet. No difference was detected in *E. coli* counts among groups with supplementation of COS at 100, 200 and 400 mg/kg or with supplementation of CTC at all 4 time points.

The immunomodulatory properties of dietary COS (0.025%) were studied in early-weaned pigs by Yin *et al.* (2008) and reported that weaning stress resulted in decreased serum antibody and cytokine levels, while dietary COS increased IL-1β gene expression in jejunal mucosa and lymph nodes, as well as serum concentrations of IL-1β, IL-2, IL-6, IgA, IgG and IgM. The authors indicated that

dietary COS may enhance the cell-mediated immune response in early-weaned piglets by modulating the production of cytokines and antibodies. Chen *et al.* (2009) evaluated the effects of diet supplementation with COS (0.50%) on immune response indicators and reported that in the post-challenge period, dietary COS decreased rectal temperature, reduced cortisol blood concentration, and increased IGF-1 concentration, but did not affect lymphocyte count. The authors concluded that dietary COS had little modulating influence on the inflammatory stress markers in weanling pigs.

Wang *et al.* (2009) reported that dietary supplementation of COS reduced the number of faecal*Escherichia coli* in growing pigs, whereas the count of faecal*Lactobacilli* was unaffected. A total of 144 [(Landrace x Yorkshire) x Duroc] pigs with an initial body weight of 23.6±1.1 kg were allotted to one of the following dietary treatments: 1) basal diet; 2) basal diet with 44 mg/kg of tylosin (100 mg/kg tylosin); 3) basal diet with 5 g/kg of COS and 4) basal diet with 5 g/kg COS and 44 mg/kg tylosin and observed that administration of COS decreased number of *E. coli* ($P<0.01$), whereas the number of faecal *Lactobacilli* was unaffected.

The goal of an experiment by Liu *et al.* (2010) was to evaluate whether COS (0.016% in the diet) can replace an antibiotic (cyadox) in reducing signs associated with infection in weaned pigs challenged with *Escherichia coli*. The obtained data did not prove the efficacy of COS as a substitute for an antibiotic growth promoter for weaners infected with *E. coli*. In challenged pigs COS increased plasma IGF-I concentrations, decreased IgA-positive cell numbers in the jejunal and ileal lamina propria, reduced the incidence of diarrhea, and alleviated

some other signs associated with infection; however, unlike cyadox, it did not alleviate the detrimental effect of the challenge on growth performance.

Yan and Kim (2011) conducted experiment using 80 commercial crossbred pigs to determine the effects of dietary inclusion of COS in weaned pigs and found reduced faecal*Escherichia coli* counts in weaned pigs fed diet supplemented with 0.30% COS.

Yang *et al.* (2012) found that dietary supplementation of COS promoted growth performance through beneficial intestine barrier function. A total of 180 weanling pigs (21 ±3 d of age; 5.98 ± 0.04 kg) were used to investigate the effect of chito-oligosaccharide (COS) on growth performance, intestinal barrier function and cecalmicroflora. Based on initialBW, gender and litter, the pigs were given 5 treatmentsduring a 14d feeding experiment, including a basaldiet (control), 3 diets with COS supplementation (200,400, or 600 mg/kg), and a diet with colistinsulphate (CSE) supplementation (20 mg/kg). Six randomlyselected pigs from each treatment were used to collectserum, duodenal, jejunal, ileal, and cecal samples on d7 and 14 postweaning. Pigs fed COS at400 mg/kg had greater (P< 0.05) concentration of *Bifidobacteria* and *Lactobacilli* in the cecum than pigsfed the control diet and CSE diet on d 7 post weaning.Supplementation of COS or CSE decreased (P<0.05) the population of caecal *Staphylococcus aureus* compared with the control diet on d 7 post weaning. The number of cecal *Bifidobacteria* in pigs fed 600 mg/kg COS was greater (P < 0.05) than that of pigs fedthe control diet or CSE diet on d 14 post weaning. Nosignificant differences were observed in *Escherichiacoli* counts in the cecum among treatments and concludedthat dietary supplementation of COS at400 or 600 mg/kg promotes growth performance andimproves gut barrier function, increases the population of

Bifidobacteria and *Lactobacilli*, and decreases *S.aureus*in the cecum of weanling pigs.

Xiao *et al.* (2013) observed that the dietary COS (0.03%) and antibiotic (chlortetracycline) supplementation had similar beneficial effects in reducing intestinal inflammation and promoting growth in weaned piglets challenged with enterotoxigenic *Escherichia coli, i.e.,* a type of bacteria which often causes post-weaning diarrhoea. Feeding with COS increased, similarly to antibiotic, concentrations of intraepithelial lymphocytes, increased villus length, villus length/crypt depth, and goblet cells, increased occluding protein and secretory IgA protein expressions, decreased Toll-like receptor 4 (TLR4) mRNA expression, and improved FCR; hence the authors concluded that chitosan has the potential to replace chlortetracycline as a feed additive for piglets.

Kong *et al.* (2014) studied the effects of dietary supplementation with COS on the gut microbiota and its metabolites using the Huanjiang mini-piglet mode and observed increased number of beneficial intestinal bacteria with suppressed growth of potential bacterial pathogens and concluded that COS presents prebiotic activity and modified the intestinal luminal environment in a presumably beneficial way in weaning Huanjiang mini-piglets.

Xiong *et al.* (2015) studied the effect of dietary supplementation of low doses of COS on intestinal morphology, immune response, antioxidant capacity and barrier function in weaned piglets and observed that the concentration of IL-10 and IgA on intestinal mucosa was higher in piglets supplemented with 30mg/kg COS, compared with unsupplemented piglets and suggested that dietary

supplementation with COS at 30mg/kg may induce an immune response in weaned piglets by modulating the production of cytokines and antibodies.

Wan *et al.* (2017) conducted experiment by using 32 healthy pigs (Landrace x Yorkshire) and observed that after dietary COS inclusion, higher ($P<0.05$) IgG concentration in the serum which indicatedthat COS can activate the intestinal mucosal immunity.

2.9 Effect of dietary chitosan supplementation on digestibility of nutrients, growth performance and efficiency of feed utilization.

Shi *et al.* (2005) supplemented the diet of broilers with increasing levels of COS (0.02-0.50%) and reported that, in comparison to control group, improved BWG and FCR, as well as nitrogen retention, was obtained by using 0.05-0.10% COS in the diet.

Han *et al.* (2007) conducted experiments with 126 crossbred weanling pigs to investigate the effect of COS on growth performance, nutrient digestibility, pH of gastro-intestinal tract, intestinal and faecalmicroflora. COS obtained from chitin of the crab shells after deacetylation and decomposition by chitosanase enzyme. Supplementation of COS at 0.30 and 0.40% levels exhibited better FCR and improved digestibility of DM and crude fat.

A total of 50 weaning pigs were selected by Liu *et al.*,(2008) to investigate the effect of dietary COS supplementation on growth performance, apparent nutrient digestibility and small intestinal morphology. Pigs housed in individual metabolic cages were assigned randomly to 5 treatments including basal diet, 3 diets with COS supplementation (100, 200 and 400 mg/kg), and 1 diet with chlortetracycline (CTC) supplementation (80 mg/kg) and reported that supplementation of COS at 100 and 200 mg/kg and supplementation of CTC

improved ($P<0.05$) overall ADG, ADFI and FCR, increased the digestibility of DM, GE, CP, Crude fat, Ca and P in comparison with control.

Wang *et al.* (2009) evaluated the effects of dietary COS on growth performance and nutrient digestibility in growing pigs. A total of 144 crossbred pigs were allotted to different dietary treatments including basal diet, basal diet with 100 mg/kg tylosin, basal diet with 5g/kg COS and basal diet with 100 mg/kg tylosin and 5g/kg COS and reported higher FCR and significant increase in digestibility of DM, nitrogen and grass energy ($P<0.05$).A study by Meng *et al.* (2010) was to evaluate the effects of diet supplementation with COS (0.02 or 0.04%) on nutrient digestibility in Hy-line Brown layers. The results showed that supplementation levels of COS had a positive influence on apparent digestibility of dry matter and nitrogen.

The results of the study with growing pigs (Yin *et al.* 2010) demonstrated that dietary COS (0.01%) enhances the net absorption of many dietary amino acids into the portal vein, so it is effective in increasing the efficiency of dietary protein utilisation by pigs.

A total of 180 weanling pigs were used by Yang *et al.* (2012) to investigate the effect of COS on growth performance. Pigs were given 5 treatments including basal diet (control), 3 diets with COS supplementation (200, 400 and 600 mg/kg), and a diet with colistin sulphate (CSE) supplementation (20 mg/kg) and reported pigs fed COS or CSE had greater ADG and ADFI compared with the control pigs.

Zhou *et al.* (2012) performed a study aimed at evaluating the effects of diet supplementation with COS on growth performance, digestibility of nutrients and diarrhoea incidence in weaned pigs and reported improved growth performance

and total tract apparent digestibility of DM and nitrogen (N) in high level inclusion of COS (2g/kg) ($P<0.05$) but both performance and digestibility were lower ($P<0.05$) than for the group supplemented with antibiotics.

Xiao *et al.* (2013) conducted experiment with 30 weaned piglets to investigate the effects of COS on growth performance and intestinal mucosal barrier function and reported feeding COS or chlortetracycline reduced ($P<0.05$) feed conversion ratio and also reported chitosan can replace chlortetracycline as a feed additive for piglets.

Xu *et al.,* (2013) evaluated the effects of chitosan on growth performance, small-intestinal morphology, and concentrations of growth hormone (GH) in the serum and of weaned pigs, in order to study the regulating action of COS on pigs growth through endocrine and intestinal morphological approaches. A total of 180 weaned pigs were selected and assigned randomly to 5 dietary treatments including 1 basal diet (control) and 4 diets with chitosan supplementation (100, 500, 1,000 and 2,000 mg/kg, respectively) and reported that the average body weight gain (BWG) of pigs was improved quadratically ($P<0.05$), may be due to increased growth hormone concentrations in the serum and ameliorated small-intestine morphology.

Xu *et al.* (2014) conducted experiments to investigate the effects of dietary chitosan on growth performance, nutrient digestibility, and digestive enzyme activities in weaned pigs. A total of 180 weaned pigs were selected and assigned randomly to 5 treatments containing 0, 100, 500, 1000 and 2000 mg chitosan per kg feed, respectively. Each treatment involved six replicate pens and six pigs per pen. On days 14 and 28, all pigs were weighed and six from each treatment (one from each replicate pen) were killed, and the contents of the stomach, jejunum,

and rectum were collected and used for determining nutrient digestibility and digestive enzyme activity. The results showed that supplementation of chitosan improved quadratically average daily gain (ADG) ($P<0.05$). Moreover, dietary chitosan quadratically ($P<0.05$) increased apparent digestibility of crude protein (CP) on days 14 and 28, and digestibility of dry matter (DM) on day 14 and of Ca and P on day 28, whereas decreased ($P<0.05$) apparent digestibility of ether extract in comparison with the control diet was observed.

A study was conducted by Swiatkiewicz *et al.* (2015) to determine the effects of chitosan on the concentrations of GH and IGF-I in serum and small intestinal morphology of piglets, in order to evaluate the regulating action of chitosan on weaned pig growth through endocrine and intestinal morphological approaches and reported that chitosan quadratically improved growth in weaned pigs and the underlying mechanism may due to the increase of the serum GH concentration and improvement of the small intestines morphology.

A study was designed by Suthongsa *et al.* (2017) to examine the effect of COS supplementation on growth performance, nutrient digestibility and small intestine functions in weaned pigs as an effective alternative to antibiotic addition in post-weaning diets. Growth, feed efficiency, small intestine morphology and crypt cell proliferation were measured at 28 and 56 days of the experiment. Pigs supplemented with 150mg/kg COS or lincomycin showed increased digestibility of crude pritein, crude fat, ash, calcium and phosphorus, increased absorption capacity on day 28 and 56 of the experiment.

Wan *et al.* (2017) demonstrated that dietary COS supplementation had a higher ($P<0.05$) apparent digestibility of crude protein, fat and dry matter than pigs in the control group. Also, the energy utilisation in the COS group was

greater ($P<0.05$) compared to the control group. In addition, the apparent digestibility of calcium and phosphorus did not differ between the treatment groups.

2.10 Effect of dietary chitosan supplementation on haematological parameters and lipid profile

Haematological parameters could serve as baseline information for comparison in conditions of nutrient deficiency, physiology and health status of farm animals. Reports by different researchers have revealed that different diets have diverse effects; either positive or negative effects on blood parameters of pigs. It thus becomes imperative to pay attention to the quality of feed fed to livestock and considerations also should be given to the influence of feed on blood parameters of farm animals, hence in the present study these characteristics were studied to evaluate the effect of dietary inclusion of SSM in pigs.

Razdan and Patterson (1994, 1996) studied the effect of inclusion of COS (0.3% in the diet) on haematological parameters and lipid profiles in broilers and found that COS decreased plasma cholesterol concentration and enhanced the ratio of HDL to total cholesterol and the authors indicated that the hypocholesterolaemic effect of dietary COS was probably connected with increased binding of bile acids by COS and consequently, with the reduction of the concentration of duodenal bile acids.

Tang *et al.* (2005) conducted a study to evaluate the effect of COS on weaned pigs indicated that feeding a diet containing 250 mg/kg COS reduces both the total cholesterol and triglyceride concentrations and further reported that the reason for this response was that COS formed a gel complex with gastric acid in the gastrointestinal tract and was subsequently excreted in the faeces. The

increased serum HDL cholesterol can carry cholesterol away from the arteries and back to the liver, subsequently slowing its buildup.

Kristy *et al.* (2007) collected a total of 361 blood samples from nursery pigs, grower/finishers and adult breeding pigs and evaluated for selected haematological parameters and serum chemistry and reported that no differences existed between males and females and also no significant difference among different age groups except lower white blood cell count and lymphocyte counts in adult breeding animals than the other age groups.

Li *et al.* (2007) found that COS (0.01%) had a positive effect on serum profile, i.e., increased, in comparison with control treatment, serum high-density lipoprotein cholesterol and decreased serum triglyceride and total cholesterol concentrations, probably through the reduction of lipid absorption in the intestine by binding bile acids, resulting in increased cholesterol elimination.

Rauw*et al.* (2009) conducted studies with 103 Duroc breed of swine to study the heritabilities and simple relationships between cholesterol and triglyceride levels and body weights. They reported that animals with high total cholesterol have high levels of HDL, LDL and serum triglycerides and the heavier animals have higher levels of total and LDL cholesterol. The mean values reported for total cholesterol, HDL, LDL cholesterol and serum triglycerides were 78.5±1.36, 32.3±0.57, 35.2±0.87 and 54.5±2.09 mg/dl, respectively

Wang *et al.* (2009) studied the effect of dietary supplementation of COS in growing pigs and reported that the RBC, WBC and albumin concentrations were not influenced by dietary treatments, but increased lymphocyte and total protein concentrations ($P<0.05$). The serum total cholesterol and triglyceride concentrations were not influenced by dietary treatments and the HDL cholesterol

concentration was higher in pigs fed diets supplemented with COS than in pigs fed diets without COS (P=0.02).

Yan and Kim (2011) reported that pigs fed COS had increased (P<0.05) lymphocyte count compared with those fed diets without COS supplementation at day 35.Results of the study with weanling pigs by Zhou *et al.*, 2012 showed that diet supplementation with 0.10 or 0.20% COS reduced blood lymphocyte concentration, without effect on erythrocyte and leukocyte concentrations.

Zhang *et al.* (2012) studied the hypolipidemic activities of high and low molecular weight chitosan in rats and found decreased levels of serum LDL-C in both high and low molecular weight chitosan groups and the mechanism in inhibiting hyperlipidemia is that chitosan could bind lipids in gastrointestinal tract and then excrete them out in feces.

Grondee*t al.* (2016) studied the mechanisms and evidence behind the hypocholesterolaemic effect of chitosanin animal trials and reported the mechanisms of chitosan action in several ways. First, where chitosan is soluble, it lowers cholesterol levels by increasing the viscosity of stomach contents, which inhibits uptake of cholesterol. Next, chitosan acts as a cationic polysaccharide in an acidic environment,e.g.the stomach, so the positive amino groups of the fibre binds to negatively charged molecules, such as bile acids and fatty acids. This leads to higher activity of the LDL-receptor and thus lowers LDL-C plasma levels. In the intestine, a higher p^H makes the complex precipitate with bound fatty and bile acids and cholesterol. After precipitation, the bound fatty and bile acids are inaccessible to enzymes and are excreted with faeces.

2.11 Effect on carcass fat and cholesterol content

Kim *et al.* (2008) reported that addition of COS in the diets of finishing pigs reduced the cholesterol in pork without affecting its quality.

2.12 Effect of dietary chitosan supplementation on carcass characteristics

Based on the earlier reports COS has a promising future as an alternative to antibiotics thereby promoting the productivity of animals, however sufficient research has not yet been done particularly in finishing pigs to know the effect of dietary supplementation of COS on carcass characteristics.

Kim *et al.* (2008) reported that inclusion of COS in the diets of finishing pigs reduced the amount of cholesterol in pork without effecting the carcass characteristics and pork quality traits, such as dressing percentage, back fat thickness, lean per cent, color and marbling score and drip losses. Two experiments were conducted to evaluate the effect of dietary lecithin with or without COS on the performance, pork cholesterol, fatty acid composition and quality of pork of finishing pigs and reported addition of COS in diets containing lecithin reduced pork cholesterol without effecting performance, carcass characters and pork quality.

Fallah and Rezaei (2013) reported that supplementation of dietary prebiotics in the broiler diets improved growth performance, carcass characters and decreased serum cholesterol level of the broiler chickens at 42 days.

CHAPTER - III

MATERIALS AND METHODS

The research work was planned to assess the utilization of shrimp shell meal as a source of prebiotic in crossbred pigs (LWY x Desi) and the biological experiment was carried out at AICRP on Pigs, Tirupati where all the facilities are available. While the laboratory analysis work was carried out at Departments of Animal Nutrition, Veterinary Microbiology, Veterinary Pathology, Veterinary Anatomy, Veterinary Physiology and Veterinary Public Health of College of Veterinary Science, Tirupati, the scanning electron microscopy was carried out at Ruska laboratory, College of Veterinary Science, SPVNRTSUVAFS, Rajendranagar, Hyderabad. In addition Fourier Transform-Infra Red Spectroscopy (FT-IR) evaluation of Chitosan was carried out at the Department of Physics, S.V. University, Tirupati.

3.1 Feed ingredients

3.1.1 Procurement of feed ingredients and shrimp shell meal

The feed ingredients like maize, soybean meal and de-oiled rice bran for preparation of standard experimental diets in ground form were procured from M/s Suvera Agencies, Chittoor and Shrimp shell meal was procured from Anjeneya shrimp processing industry in Nellore district of Andhra Pradesh.

3.1.2 Analysis of feed ingredients and shrimp shell meal

Representative samples of feed ingredients and creep, grower and finisher diets were analyzed for proximate composition as per AOAC (2005). Shrimp shell meal obtained from the shrimp processing unit contained about 30% DM. Hence, it was sundried for 3 days to get about 90% DM, ground in a hammer mill and sterilized in a autoclave for 10 min at 121°C and 15psi

pressure. A representative sample was analyzed for proximate composition (AOAC, 2005), cell wall constituents (Van Soest *et al.* 1973) and Ca and P (Talapatra *et al.*1940).

3.1.3 Chitosan extraction

Chitosan extraction and characterization was carried out to estimate the yield of chitosan from shrimp waste meal.

3.1.3.1 Demineralization

The ground shrimp shell meal was demineralised using 1% HCl in 1:4 ratio (Puvvada *et al.* 2012). It is required to soak the sample for 24 h to remove the minerals (mainly calcium carbonate) (Trung *et al.* 2006) and treated for one hour with 50 ml of 2% NaOH solution to decompose the albumen into water soluble amino-acids. The resulting residue was chitin, which was washed with deionized water and the water then drained off.

3.1.3.2 Deacetylation

The deacetylation was carried out by adding 50% NaOH to the extracted chitin and boiled at 100°C for 2 h on a hot plate. The samples were cooled for 30 minutes to room temperature, washed continuously with 50% NaOH solution and filtered to retain the solid matter, which was the chitosan. Chitosan was oven dried at 110°C for 6 h and characterized by Fourier Transform-Infra Red Spectroscopy (Puvvada *et al.* 2012). A sample (10µg) was mixed with 100 µg of dried Potassium bromide and compressed to prepare a salt (10 mm diameter) and characterized by the FT-IR in the range of 400-4000 cm^{-1}.

3.2 *In vivo* experiment

3.2.1 Experimental diets

The study was conducted during pre-weaning, grower (two weeks after weaning to 35 kg) and finisher (35 to70 kg) stages in crossbred pigs.

During the pre-weanig stage the following treatments were evaluated using 6 litters per treatment.

T1: Negative control group

T2: Creep feed with antibiotic (positive control)

T3: Creep feed with 0.5% shrimp shell meal

T4: Creep feed with 1.0% shrimp shell meal

T5: Creep feed with 1.5% shrimp shell meal

During the grower stage (two weeks post weaning to 35 kg BW) the following treatments were evaluated using 8 pigs / treatment.

T1 : Negative control group

T2: grower feed with antibiotic (positive control)

T3: grower feed with 2. 5 % shrimp shell meal

T4: grower feed with 5.0 % shrimp shell meal

T5: grower feed with 7.5 % shrimp shell meal

During the finisher stage (35 kg -70 kg BW) the following treatments were evaluated using 6 pigs / treatment.

T1 : Negative control group

T2: Finisher feed with antibiotic (positive control)

T3: Finisher feed with 2.5 % shrimp shell meal

T4: Finisher feed with 5.0 % shrimp shell meal

T5: Finisher feed with 7.5 % shrimp shell meal

3.2.2 Experimental design

The following parameters were studied during all the three stages of growth

- ADG, EFU and cost of feed/kg gain during pre weaning, grower and finisher stages.
- A metabolism trial during finisher stage to study the effect of experimental rations on nutrient digestibility and nitrogen balance.

Two piglets per treatment at the time of weaning, 2 pigs per treatment at the end of grower phase and 6 pigs per treatment at the end of finisher phase *i.e.* 70 kg live weight were slaughtered to study the following parameters:

- Small intestinal morphology *i.e.* villi height and crypt depth.
- Hematological characteristics (Albumin, Total protein, IgG, WBC, RBC, Lymphocyte)
- Effects of dietary chitosan supplementation on serum lipid profile (Total cholesterol, HDL, LDL and Triglycerides)
- Gut pathogens (*E.coli* and *Salmonella*).
- Carcass fat and cholesterol content after weaning and grower stages
- Carcass traits such as length, dressing percentage, loin eye area, back fat thickness, carcass fat and cholesterol content at the end of study period.

3.3 Growth trial

Before intiation of the growth trial all the experimental procedures were reviewed and approved by the animal ethics committee of the College of Veterinary Science, Tirupati. The experimental rations were formulated as per NRC, 1998.

3.3.1 Creep phase (10-42 days)

The dietary treatments (T1-T5) were evaluated using six litters per treatment and the number of piglets were 50, 53, 52, 54, and 51 with an initial

body weight (kg) of 1.37±0.04, 1.31±0.02, 1.12±0.01, 1.17±0.01 and 1.35±0.03 in treatments 1 to 5, respectively.

3.3.2 Grower phase (Two weeks post-Weaning to 35.0 kg body weight)

At the end of creep phase, eight piglets from each treatment were selected as and when they reached 15.0 kg body weight dewormed and fed on the grower diets till they attained 35 kg body weight. Daily feed offered and left over was recorded and the body weights of pigs were recorded every week. Two piglets per treatment were slaughtered after grower phase to study intestinal morphology and gut pathogen load.

3.3.3 Finisher phase (35.0 kg body weight – 70 kg body weight)

The remaining six pigs were shifted to corresponding finisher (35-70 kg) diets as and when they attained 35 kg to evaluate their growth performance.

The pigs were housed individually in separate pens during both grower and finisher phases. All the pigs were dewormed before the start of the grower and finisher stages and had free access to feed and water throughout the growth trial. The daily feed offered and the left over were recorded and the body weight of the pigs were recorded every week.

3.4 Metabolism trial

A metabolism trial was conducted during finisher phase using all the six pigs in each treatment by housing them individually in metabolic cages with free access to water and feed. The pigs were acclimatized to the cages for 3 days followed by a collection period of 5 days. During the collection period, the daily feed offered, left over feed, faeces and urine voided were recorded. About 50 ml of sulphuric acid (laboratory grade) was added to the urine container to prevent nitrogen loss and an aliquot of $1/10^{th}$ of the total faeces voided and $1/100^{th}$ of the

total urine output were preserved for further laboratory analysis and the urine was frozen at -20°C to estimate the N, Ca, and P contents.

3.5 Study of small intestinal morphology

Two piglets per treatment at the end of creep and grower phase and 6 pigs per treatment at the end of finisher phase were slaughtered to study the intestinal morphological feachers. The small intestine was excised immediately after slaughter and approximately 2 cm segments of the duodenum, jejunum and ileum were taken from the middle of each part, cut transversely, washed with physiological saline and 2 mm^2 tissue samples were cut from these sections and submerged in a fixative solution containing 2.5% buffered glutaraldehyde for scanning electron microscope study. The remaining portion was immersed in 10% buffered formalin.

Scanning Electron Microscopy (SEM) was performed on the 2 mm^2 specimens. Samples were fixed in 2.5% gluteraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 hours at 4°C and post fixed in 2% aqueous osmium tetroxide for 4 h. Then dehydrated in series of graded alcohols and dried to critical point drying with CPD unit. The processed samples were mounted over the stubs with double-sided carbon conductivity tape and a thin layer of gold coat over the samples were done by using an automated sputter coated (Model – JEOL JFC-1600) for 3 minutes and scanned under Scanning Electron Microscope (SEM – Model: JOEL-JSM 5600) at required magnifications as per the standard procedures (Bozzola and Russell, 1998) at RUSKA Laboratory, College of Veterinary Science, Rajendranagar, Hyderabad and were photographed.

Cross-sectional small intestine samples from the formalin preserved segments were cut to approximately 5 µm thickness and stained with

haematoxylin and eosin (Nabuurs *et al.* 1993). Well-oriented crypt-villus units were selected for each intestinal cross-section and villus height and the crypt depth were measured at 10X magnification using a light microscope equipped with an ocular micrometer (Li *et al.*1990) and ratio of villus to crypt depth was calculated. Villus height was measured from the tip of the villus to villus-crypt junction and crypt depth was defined as the depth of the invagination between adjacent villi (Hou *et al.* 2006).

3.6 Estimation of hematological characteristics

Blood samples were collected from the experimental animals at the time of slaughter into vacuum tubes containing no additive and tubes containing K_3 EDTA to obtain serum and whole blood, respectively. The red blood cells, white blood cells and lymphocyte counts of whole blood samples were determined using an automatic blood analyzer (ADVIA 120, Bayer, Tarrytown, NY, USA). The serum was separated by centrifugation for 30 minutes at 2000 x g at 4°C, the aliquot was stored at -4°C for determination of serum profiles.

3.7 Estimation of serum lipid profile

The total cholesterol, HDL and the triglycerides in the serum samples were estimated using diagnostic kits (M/s. Span Diagnostics Private Limited) by enzymatic method of Allian (1974), PEG precipitation and enzymatic method of Wiebs and Smith (1985) and enzymatic methods of Gowan (1983), respectively. The LDL cholesterol was calculated as the difference between total cholesterol and HDL cholesterol. Serum total proteins and albumin were estimated using diagnostic kit (Monozyme India Limited) as described by Gornall *et al.,* (1949) and globulin was estimated by subtracting albumin from the total protein.

3.8 Collection of gut contents

A portion of large intestine between caecum and colon measuring 25cm length was ligated on both the sides and it was cut behind the ligation on both the sides with a sterilized knife. The collected intestine piece was placed in a sterilized beaker and kept at refrigeration temperature (4±1°C) to estimate gut pathogens (American Public Health Association, 1984).

3.8.1 Estimation of gut pathogens

One gram of the above intestinal content was mixed with 9 ml of sterile nutrient broth. Serial dilutions of each sample were prepared and the samples in duplicates were incubated at 37°C over a period of 24-48 h. After incubation, one ml of the inoculum was transferred into petri plates containing EMB agar and BGA agar that are specific for *E.coli* and *Salmonella* and the inoculum was uniformly streaked throughout the plate by surface spread method. The plates meant for total counts were incubated at 37°C over a period of 24-48 h. Those plates revealing visible colonies in the range of 30-300 were selected, counted and the counts were expressed as $\log_{10}$ cfu/g of sample.

3.9 Estimation of muscle cholesterol

The longissimus dorsi muscle sample from each slaughtered experimental animal was collected and preserved at –20°C for estimation of muscle cholesterol and triglyceride content**.**

Lipid from the muscle tissue was extracted according to the procedure of Folch *et al.* (1957). Two grams of muscle tissue was homogenized in 7ml of methanol using Teflon homogenizer. The contents were filtered with the help of Whatman filter paper (No.40). The contents on filter paper were scrapped off and homogenized with 14 ml of chloroform – methanol mixture and filtered into a flask. The residue was successively homogenized in chloroform – methanol (2:1

v/v) and each time this extract was filtered and it was repeated for two times. The pooled filtrate was evaporated to dryness.

The dried residue was dissolved in 5ml of chloroform – methanol mixture (2:1 v/v) and transferred into a centrifuge tube. To the above, 2ml of 0.1 M potassium chloride was added, shaken well and centrifuged at 3000RPM for 10 minutes. The upper aqueous layer containing gangliosides was discarded and the chloroform layer was mixed with 1 ml of chloroform – methanol – potassium chloride mixture (1:10:10 v/v) and centrifuged again. The washing was repeated thrice and each time, the upper layer was discarded. The remaining mixture was evaporated to dryness and the amounts of total lipids were estimated gravimetrically. The lower layer was made up to 5ml with chloroform – methanol mixture (2:1 v/v) and used for the analysis of lipid profile. The total cholesterol was estimated by enzymatic method using standard kits.

3.10 Carcass characteristics

When the experimental animals attain about 70 kg, body weight they were slaughtered (USDA, 1970) after 16 h fasting period but with *ad lib* access to water. The dressing percentage was calculated from half carcass weight with intact kidneys and also with head and feet on. Loin area was traced on acetate paper by keeping it between 10^{th} and 11^{th} ribs. The traced area was measured in square centimeters. The back fat thickness was measured at three locations *i.e.*, first rib, last rib and the last lumbar vertebra and the average was reported as average back fat thickness.

At the time of slaughter, 25 cm of large intestine between caecum and colon was ligated on both sides and it was cut by a sterilized knife and the same

was collected in a sterilized potato tube by following all the aseptic precautions and it was brought to the laboratory for microbiological analysis.

3.11 Statistical analysis

The data were subjected to one –way analysis of variance (Snedecor and Cochran, 1989) and the means were tested by least significant difference. The data obtained by slaughter of only two pigs at the end of creep and grower phase were subjected to one way ANOVA from summary data.

CHAPTER – IV

RESULTS

The results of present study are presented in this chapter. The chemical composition of shrimp shell meal, characterization ofchitosan, nutrient composition of feed ingredients,ingredient and chemical composition of experimental diets, performance of animals fed experimental diets in terms of body weight gain, feed intake and feed efficiency are presented in this chapter.Further, effect of experimental diets on the digestibility (%) of various nutrients, small intestine morphological features, haematological characters, gut pathogens, carcass fat and cholesterol content, carcass characteristics, serum lipid profile and economics of production are also presented in this chapter.

4.1 Chemical composition of shrimp shell meal

The chemical composition (%) of shrimp shell meal (Table 2) revealed that it contained 29 (DM), 39.5 (CP), 4.8 (EE), 8.7 (CF), 24.8(TA), 22.2(NFE),7.18 (Ca), 3.45 (P), 55.70 (NDF), 53.26 (ADF), 1.29 (cellulose), 0.82 (ADL) and 6.92 (silica). It also contained 15.5% chitosan estimated after deacetylation of chitin.

Table 2: The chemical composition (% DM) and chitosan (%) of shrimp shell meal

Moisture	71.0
CP	39.5
EE	4.8
CF	8.7
TA	24.8
NFE	22.2
NDF	55.70
ADF	53.26
Cellulose	1.29
ADL	0.82
Silica	6.92
Chitosan	15.5
Calcium	7.18
Phosphorus	3.45

4.2 Characterization of chitosan

The chitosan extracted from shrimp shell meal was characterized using the Fourier Transform-Infra Red spectroscopy (FT-IR) (Table 3 and Fig. 1 & 2). The FT-IR of extracted chitosan from shrimp shell meal showed peak at 3851 cm^{-1} representing presence of OH group. Similarly peaks of amide bands 1, 2 and 3 were observed at 1628, 1548 and 1373 cm^{-1} whichcompared well with amide peaks at 1643, 1552 and 1374 cm^{-1} for standard chitosan.The NH stretching peak of the extracted chitosan was at 3432 cm^{-1} which compared well with the NH stretching peak of 3609 cm^{-1} for the standard chitosan. Presence of the entire band stretching in the extracted chitosan compared with the standard chitosan band stretching showed that the extracted material was chitosan.

Table 3: Main bonds observed in the FT-IR spectra of standard chitosan and extracted chitosan of the present study from shrimp shell meal

Vibration mode (Pearson *et al.*, 1960)	Std. Chitosan (cm^{-1}) (Puvvada *et al*., 2012)	Chitosan extracted from shrimp shell meal of the present study (cm^{-1})
OH stretching	3858	3851
NH stretching	3609	3432
CH stretching	2862	2882
Amide band	1643	1628
Amide 2 band	1552	1548
CH2 bending and CH3 deformation	1421	1426
Amide 3 band and CH2 wagging	1374	1373
Asymmetric bridge O2 stretching	1313	1309
CO- stretching	1022	1008
CH3 wagging alone chain	752	697
NH-out of plane bending	564	621

Fig.1 : FT-IR spectrum of extracted chitosan from shrimp shell meal

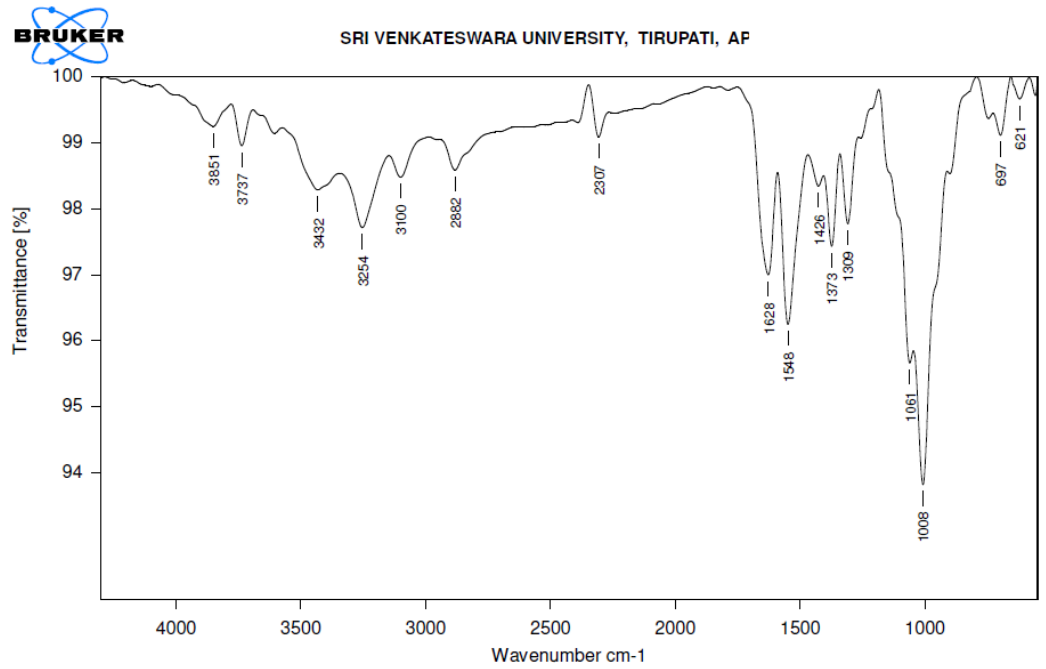
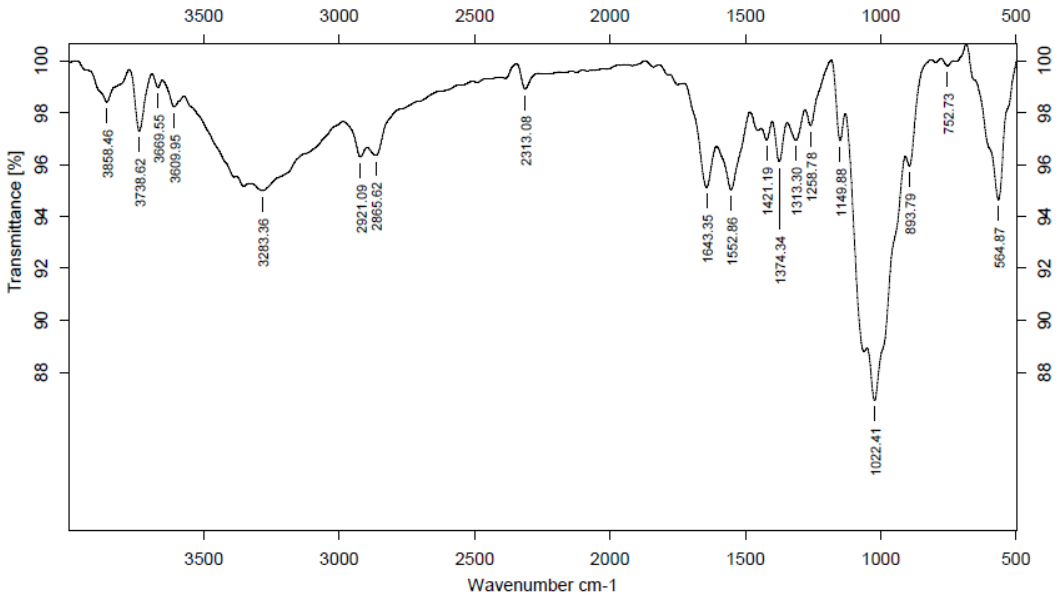


Fig.2: FT-IR spectrum of standard chitosan (Puvvada *et al.*, 2012)



4.3Nutrient composition of feed ingredients

The nutrient content (%DM) of maize, soybean meal, shrimp shell meal and de-oiled rice bran was 9.89, 44.45, 39.5 and 12.78 (CP); 1.85, 1.23, 4.8 and 0.52 (EE); 2.87, 6.66, 8.7 and 17.93 (CF); 1.94, 7.72, 24.8 and 17.98 (TA), respectively. The Ca and P content (%) was 0.01 and 0.13 (maize); 0.20, 0.37 (SBM); 7.18, 3.45 (SSM) and 0.06, 0.8 (DORB), respectively. The lysine and methionine content estimated from CP of the ingredients were 0.28 and 0.21 (maize), 2.50 and 0.53(SBM), 6.17 and 2.84(SSM) and 0.31 and 0.22 (DORB), respectively.

4.4 Ingredient and chemical composition of the experimental diet

4.4.1. Creep diets

The ingredient and chemical composition (%) of experimental creep diets fed to piglets from 10 to 42 days is presented in Table 4. The diets were formulated as per NRC, 1998 requirements. The standard diet contained maize, soybean meal and deoiled rice bran.

The ingredient composition (%) of the experimental diets was 65.0 (maize), 6.0 (deoiled rice bran), 2.0 (mineral mixture), 0.5 (salt), 26.5, 26.5, 26.0, 25.5, 25.0 (soyabean meal) and 0, 0, 0.5, 1.0, 1.5 (shrimp shell meal) for T1 to T5, respectively.The diets contained 89.56, 90.12, 90.17, 90.28 and 90.32% DM; 91.79, 91.69, 90.48, 90.11 and 89.99% OM; 19.93, 19.87, 19.86, 20.02 and 19.98% CP; 8.21, 8.31, 9.52, 9.89 and 10.01% TA; 2.01,2.11, 1.67,1.86 and 1.98% EE; 5.26, 5.21, 7.01, 7.32 and 7.69% CF and 64.59, 64.50, 61.94, 60.91 and 60.34 % NFE for T1 to T5 , respectively.

Table 4: Ingredient and chemical composition (%) of experimental creep diets

Ingredient	T1 (NC)	T2 (PC)	T3	T4	T5
Maize	65.0	65.0	65.0	65.0	65.0
Deioled Rice Bran	6.0	6.0	6.0	6.0	6.0
Soybean meal	26.5	26.5	26.0	25.5	25.0
Mineral Mixture	2.0	2.0	2.0	2.0	2.0
Salt	0.5	0.5	0.5	0.5	0.5
Shrimp shell meal	-	-	0.5	1.0	1.5
	100	**100**	**100**	**100**	**100**
Chlorotetracycline	-	0.08	-	-	-
Lysine	0.98	0.98	1.02	1.05	1.21
Methionine	0.58	0.58	0.60	0.62	0.64
AB_2D_3	0.02	0.02	0.02	0.02	0.02
Chemical composition (%) [a]					
DM	89.56	90.12	90.17	90.28	90.32
OM	91.79	91.69	90.48	90.11	89.99
CP	19.93	19.87	19.86	20.02	19.98
TA	8.21	8.31	9.52	9.89	10.01
EE	2.01	2.11	1.67	1.86	1.98
CF	5.26	5.21	7.01	7.32	7.69
NFE	64.59	64.50	61.94	60.91	60.34
Calcium	0.89	0.91	0.95	1.01	1.08
Total phosphorus	0.69	0.68	0.74	0.81	0.90
DE (kcal/kg)*	3200	3200	3175	3150	3125

[a] on Dry Matter basis

***Calculated

4.4.2. Grower diets

The ingredient and chemical composition (%) of the experimental grower diets fed to pigs from 15kg to 35 kg body weight is presented in Table 5. The diets were formulated as per NRC, 1998 requirements. The standard diet contained maize, soybean meal and deoiled rice bran.

The ingredient composition (%) of experimental diets was 55.0, 55.0, 55.0, 55.0 and 55.0 (maize), 17.5, 17.5, 16.0, 16.5 and 16.0(deoiled rice bran), 25.0, 25.0, 24.0, 21.0 and 19.0 (soybean meal) and 0, 0, 2.5, 5.0 and 7.5 (shrimp shell meal) for T1to T5, respectively.

The diets contained 89.16, 89.23, 90.19, 90.34 and 90.45% DM; 88.98, 88.99, 87.76, 87.14 and 87.04% OM; 18.05, 18.05, 18.41, 18.12 and 18.16 % CP; 11.02, 11.01, 12.24, 12.86 and 12.96% TA; 1.85, 1.85, 2.03, 2.21 and 2.54% EE; 6.91, 6.87, 9.12, 10.45 and 11.02% CF and 62.17, 62.22, 58.20, 56.36 and 55.32% NFE for T1 to T5, respectively.

Table 5: Ingredient and chemical composition (%) of experimental grower diets

Ingredient	T1 (NC)	T2 (PC)	T3	T4	T5
Maize	55.0	55.0	55.0	55.0	55.0
Deioled Rice Bran	17.5	17.5	16.0	16.5	16.0
Soybean meal	25.0	25.0	24.0	21.0	19.0
Shrimp shell meal	-	-	2.5	5.0	7.5
Mineral Mixture	2.0	2.0	2.0	2.0	2.0
Salt	0.5	0.5	0.5	0.5	0.5
	100	**100**	**100**	**100**	**100**
Chlorotetracycline	-	0.080	-	-	-
Lysine	0.76	0.76	0.78	0.81	0.85
Methionine	0.32	0.32	0.41	0.44	0.48
AB_2D_3	0.02	0.02	0.02	0.02	0.02
Cost per 100 kg (Rs)	22.50	22.50	20.98	20.14	19.0
Chemical composition (%) [a]					
DM	89.16	89.23	90.19	90.34	90.45
OM	88.98	88.99	87.76	87.14	87.04
CP	18.05	18.05	18.41	18.12	18.16
TA	11.02	11.01	12.24	12.86	12.96
EE	1.85	1.85	2.03	2.21	2.54
CF	6.91	6.87	9.12	10.45	11.02
NFE	62.17	62.22	58.20	56.36	55.32
Calcium	0.64	0.64	0.66	0.68	0.70
Total phosphorus	0.72	0.72	0.74	0.74	0.74
Available phosphorus	0.14	0.14	0.16	0.18	0.22
DE (kcal/kg)*	3100	3100	3100	3100	3100

[a] on Dry Matter basis

***Calculated

4.4.3. Finisher diets

The ingredient and chemical composition (%) of the experimental finisher diets fed to pigs from 35kg to 70 kg body weight is presented in Table 6. The diets were formulated as per NRC, 1998 requirements. The standard diet contained maize, soybean meal and deoiled rice bran.

The ingredient composition (%) of the experimental diets was 58.75, 58.75, 58.0, 61.0 and 60.0 (maize), 20.0, 20.0, 20.25, 16.75 and 17.50 (deoiled rice bran), 19.0, 19.0, 17.0, 15.0 and 12.75 (soybean meal) and 0, 0, 2.5, 5.0 and 7.5 (shrimp shell meal) for T1 to T5, respectively.

The diets contained 89.62, 89.61, 89.89, 90.18 and 90.36 % DM; 88.31, 88.31, 87.98, 87.52 and 87.04% OM; 16.05, 16.05, 16.11, 16.05 and 16.04 % CP; 11.69, 11.69, 12.02, 12.48 and 12.96 % TA; 1.09, 1.09, 1.69, 1.92 and 1.97 % EE; 10.21, 10.21, 11.05, 12.12 and 12.32 % CF; and 60.96, 60.96, 59.13, 57.43 and 56.74 % NFE for T1 to T5, respectively.

Table 6: Ingredient and chemical composition (%) of experimental finisher diets

Ingredient	**T1 (NC)**	**T2 (PC)**	**T3**	**T4**	**T5**
Maize	58.75	58.75	58.0	61.00	60.0
Deioled Rice Bran	20.00	20.00	20.25	16.75	17.50
Soybean meal	19.00	19.00	17.00	15.00	12.75
Mineral Mixture	2.0	2.0	2.0	2.0	2.0
Salt	0.25	0.25	0.25	0.25	0.25
Shrimp shell meal	-	-	2.50	5.0	7.5
	100	**100**	**100**	**100**	**100**
Chlorotetracycline	-	0.080	-	-	-
Lysine	0.67	0.67	0.72	0.75	0.76
Methionine	0.24	0.24	0.26	0.28	0.31
AB_2D_3	0.02	0.02	0.02	0.02	0.02
Cost per 100 kg (Rs)	20.50	20.75	19.30	18.45	17.75
Chemical composition (%) [a]					
DM	89.62	89.61	89.89	90.18	90.36
OM	88.31	88.31	87.98	87.52	87.04
CP	16.05	16.05	16.11	16.05	16.04
TA	11.69	11.69	12.02	12.48	12.96
EE	1.09	1.09	1.69	1.92	1.97
CF	10.21	10.21	11.05	12.12	12.32
NFE	60.96	60.96	59.13	57.43	56.71
Calcium	0.64	0.64	0.66	0.68	0.71
Total phosphorus	0.72	0.72	0.74	0.74	0.76
Available phosphorus	0.15	0.15	0.16	0.16	0.16
DE (kcal/kg)*	3100	3100	3100	3100	3100

[a] on Dry Matter basis

***Calculated

4.5. Effects of experimental diets on small intestinal morphological feachers

4.5.1. In piglets (pre-weaning animals)

The effect of feeding different treatment diets on the small intestinal morphological feachers in pre-weaned piglets is presented in Table 7 and Fig. 3-8. The height of villi (µm) in duodenum of pigs fed on T4 ration was significantly the highest ($P<0.01$), while in T3 and T5 fed pigs it was significantly higher ($P<0.01$) than in T1 and T2 and the values were 166.8, 177.4, 217.8, 393.4 and 315.2 for T1 to T5 fed pigs, respectively. The crypt depth (µm) in duodenum of pigs fed on T2 was significantly lowest ($P<0.01$). The crypt depth in T3 and T4 is comparable and higher than the control. The values were 176.8, 140.6, 189.8, 188.8 and 267.0 for T1 to T5 fed pigs, respectively. The ratio of villus height to crypt depth (VH/CD) in duodenum was highest ($P<0.01$) in pigs fed T4 ration and comparable among T1, T2, T3 and T5 and the values were 0.94, 1.26, 1.14, 2.08 and 1.18 for T1 to T5 fed pigs, respectively.

The villi height in jejunum was significantly lower ($P<0.01$), in T1 fed pigs and the values were 171.0, 243.0, 245.0, 254.2 and 246.6 for T1 to T5 fed pigs, respectively. The crypt depth in jejunum of pigs fed on T4 ration was significantly lower ($P<0.01$) than in other treatments except control and the values were 166.4, 192.0, 225.2, 122.0 and 222.2 for T1 to T5 fed pigs, respectively. The ratio of villus height to crypt depth (VH/CD) in jejunum was the highest ($P<0.01$) in pigs fed T4 ration and villus height to crypt depth ratio was significantly higher ($P<0.01$) in T2, T3 and T5 fed pigs than T1 and the values were 1.02, 1.27, 1.08, 2.08 and 1.12 for T1 to T5 fed pigs, respectively.

The height of villi in ileum of pigs fed T4 ration was significantly the highest ($P<0.01$) and comparable in pigs fed T1, T2 and T3 and the values were 174.4, 184.4, 177.6, 247.4 and 226.8 for T1 to T5 fed pigs, respectively. The crypt depth in ileum of pigs fed T4 was significantly the lowest ($P<0.01$), while the crypt depth of ileum of pigs fed T1, T2 and T3 were comparable among treatments and the values 178.6, 170.8, 176.0, 140.2 and 211.6 in pigs fed T1 to T5, respectively. The ratio of villus height to crypt depth (VH/CD) in ileum is significantly wide ($P<0.01$) in pigs fed T4 ration. The ratio of villus height to crypt depth was in order of T2>T5> T3>T1 fed pigs and the difference was significant ($P<0.01$). The values were 0.97, 1.08, 1.01, 1.76 and 1.06 for T1 to T5 fed pigs, respectively.

Table 7: Effect of experimental diets on small intestinalmorphological structure in pre-weaned piglets[1]

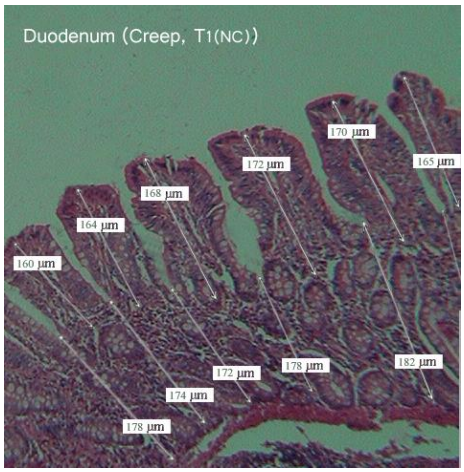
Item		Treatments				
		T1 (NC)	**T2 (PC)**	**T3**	**T4**	**T5**
Duodenum	Villus height (µm)	166.8^{d}±2.15	177.4^{d}±3.69	217.8^{c}±2.90	393.4^{a}±1.72	315.2^{b}±9.09
	Crypt depth (µm)	176.8^{c}±1.74	140.6^{d}±3.81	189.8^{b}±5.26	188.8^{b}±2.06	267.0^{a}±5.51
	VH/CD[2]	0.94^{c}±0.01	1.26^{b}±0.02	1.14^{b}±0.04	2.08^{a}±0.02	1.18^{b}±0.04
Jejunum	Villus height (µm)	171.0^{c}±2.82	243.0^{b}±1.70	245.0^{b}±2.30	254.2^{a}±1.35	246.6^{b}±2.15
	Crypt depth (µm)	166.4^{c}±3.72	192.0^{b}±10.15	225.2^{a}±2.08	122.0^{d}±1.37	222.2^{a}±10.58
	VH/CD[2]	1.02^{c}±0.01	1.27^{b}±0.07	1.08^{c}±0.01	2.08^{a}±0.02	1.12^{c}±0.06
Ileum	Villus height (µm)	174.4^{c}±0.50	184.4^{c}±1.46	177.6^{c}±1.72	247.4^{a}±1.16	226.8^{b}±12.81
	Crypt depth (µm)	178.6^{b}±0.67	170.8^{b}±0.58	176.0^{b}±1.41	140.2^{c}±4.99	211.6^{a}±6.80
	VH/CD[2]	0.97^{c}±0.01	1.08^{b}±0.01	1.01^{b}±0.01	1.76^{a}±0.05	1.06^{b}±0.02

1 The number of observations for each mean value was five (n=5)

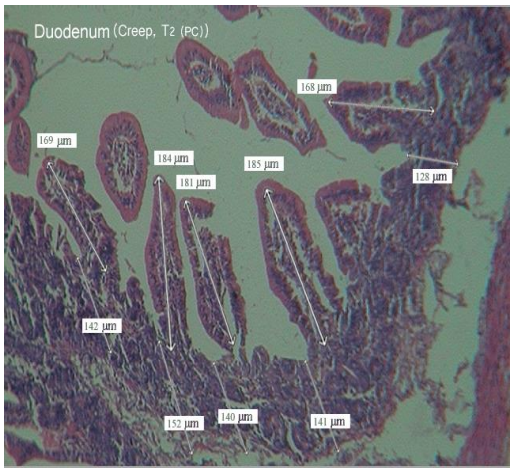
2 VH/CD means the ratio of villus height to crypt depth

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

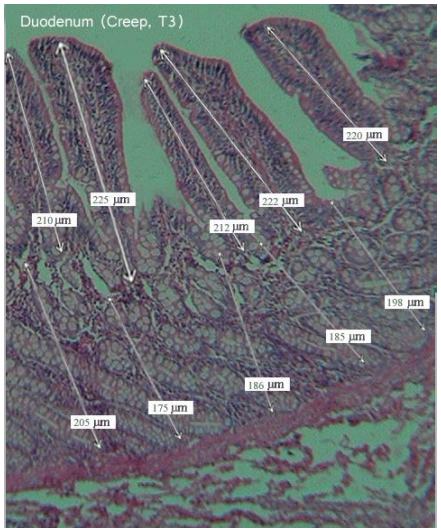
Fig.3: Photomicrograph of small intestine (Duodenum) of pre-weaned piglets showing villi height and crypt depth (Hematoxylin and Eosine x 10X)



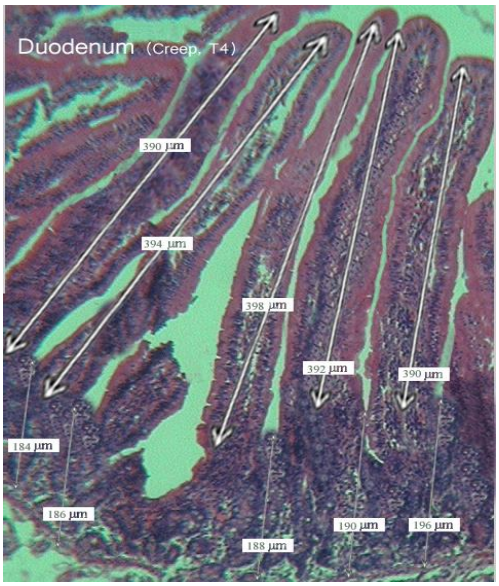
T1 (NC)



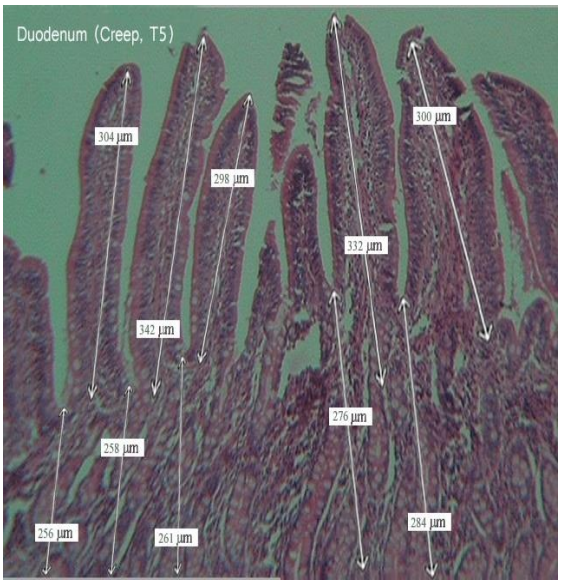
T2 (PC)



T3

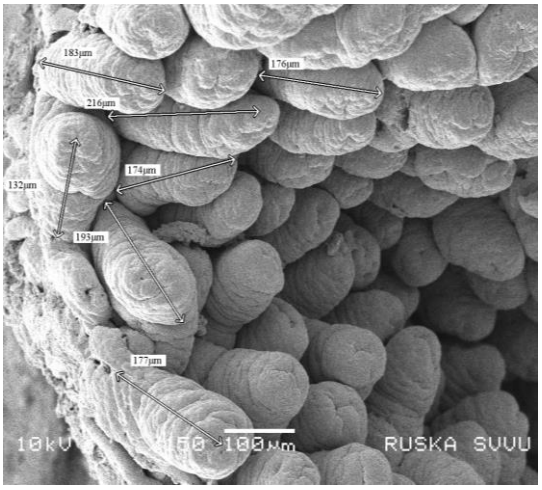


T4

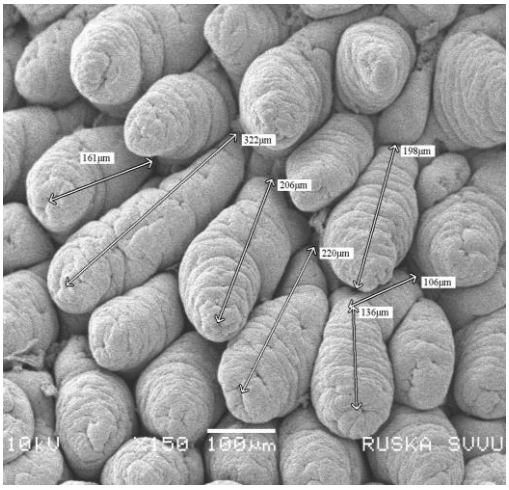


T5

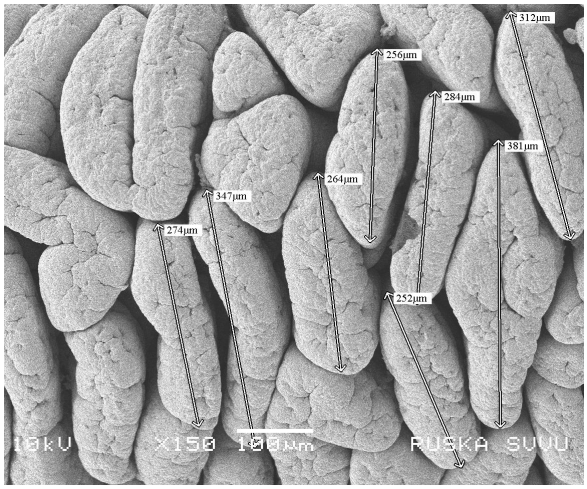
Fig.4 : Electron-micrographs of small intestine (Duodenum) of pre-weaned piglets showing villi height (SEM images)



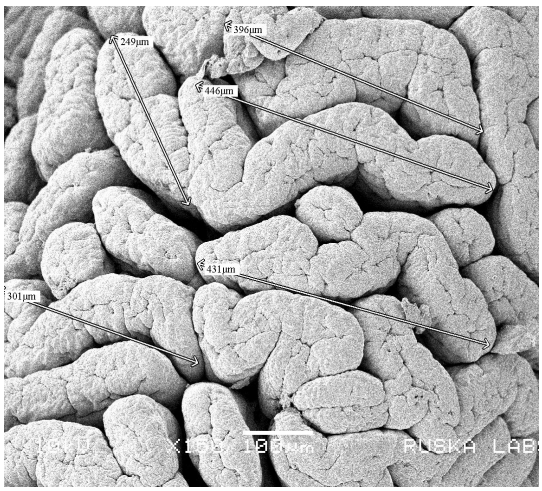
T1 (NC)



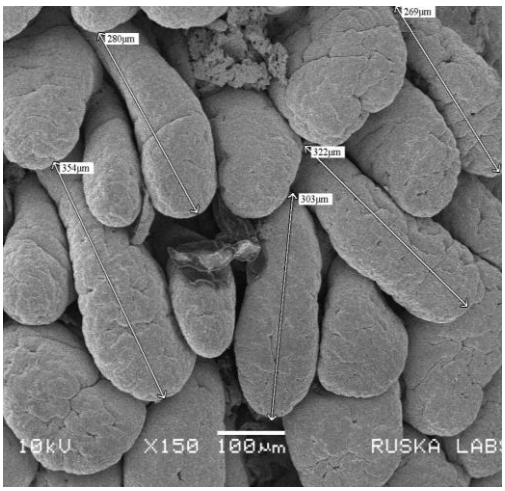
T2 (PC)



T3

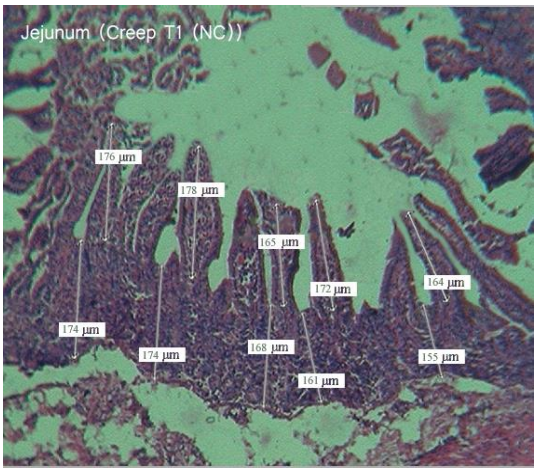


T4

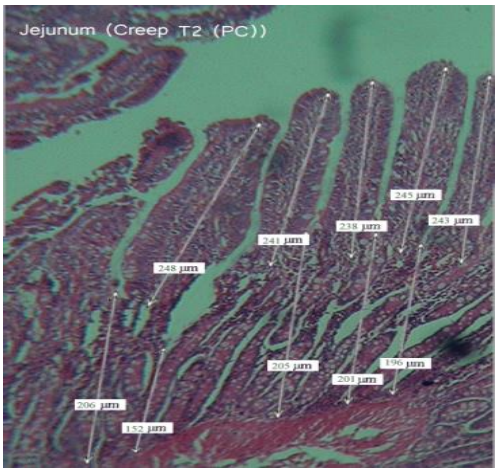


T5

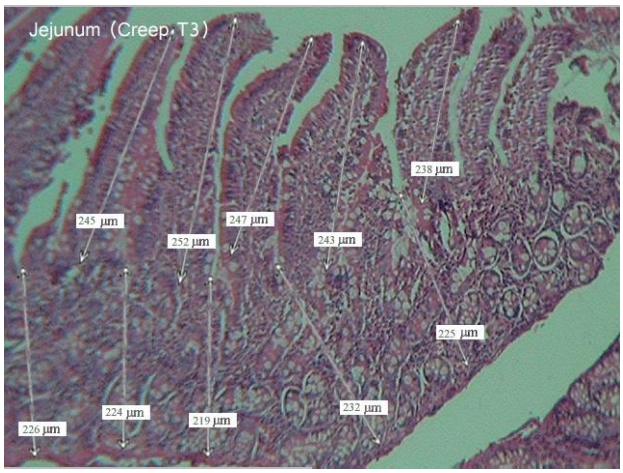
Fig.5: Photomicrograph of small intestine (Jejunum) of pre-weaned piglets showing villi height and crypt depth (Hematoxylin and Eosine x 10X)



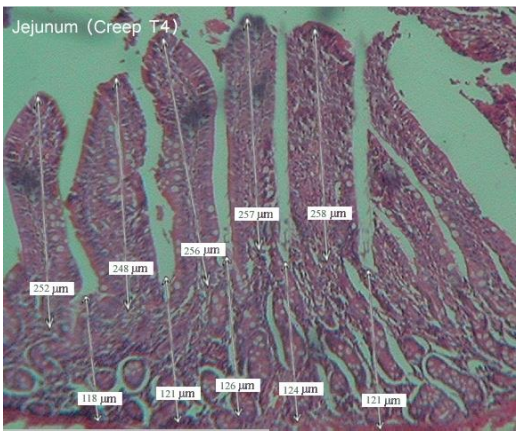
T1 (NC)



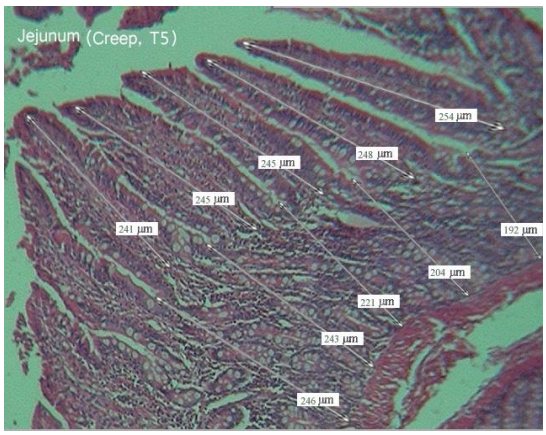
T2 (PC)



T3

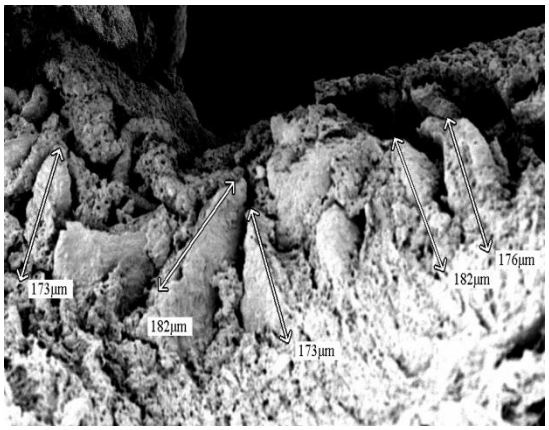


T4

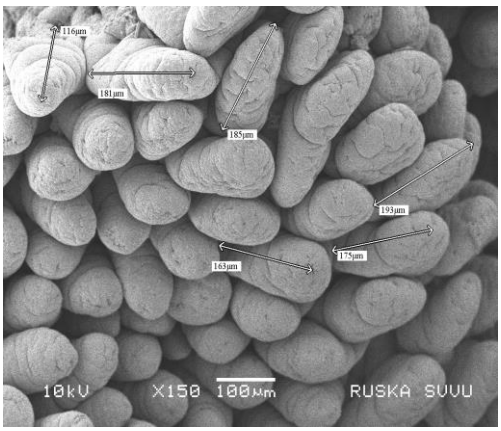


T5

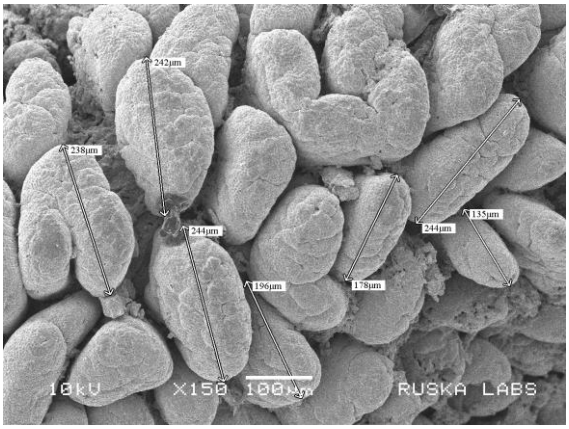
Fig.6: Electron-micrographs of small intestine (Jejunum) of pre-weaned piglets showing villi height (SEM images)



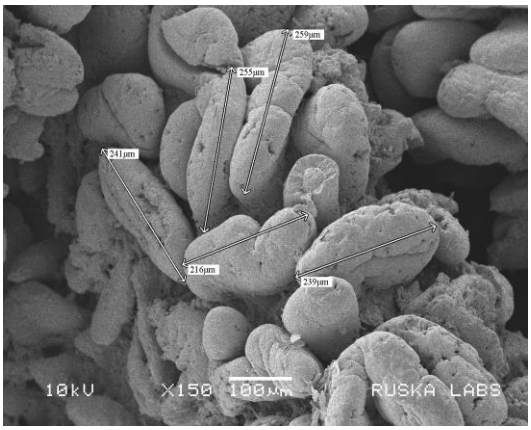
T1 (NC)



T2 (PC)



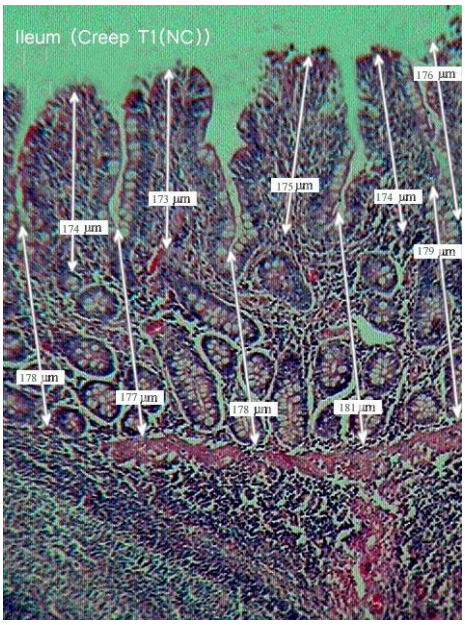
T3



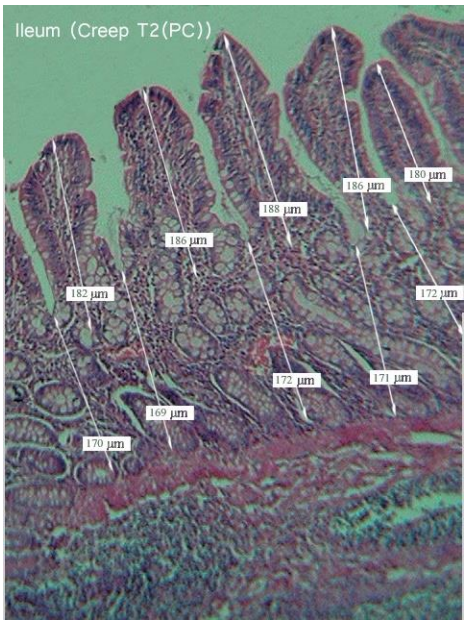
T4

T5

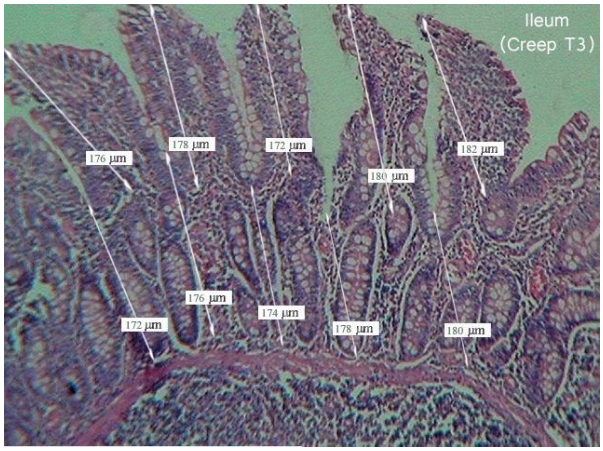
Fig.7: Photomicrograph of small intestine (Ileum) of pre-weaned piglets showing villi height and cryptdepth (Hematoxylin and Eosine x 10X)



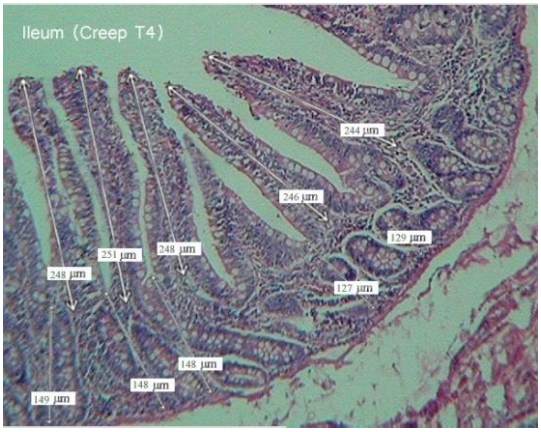
T1 (NC)



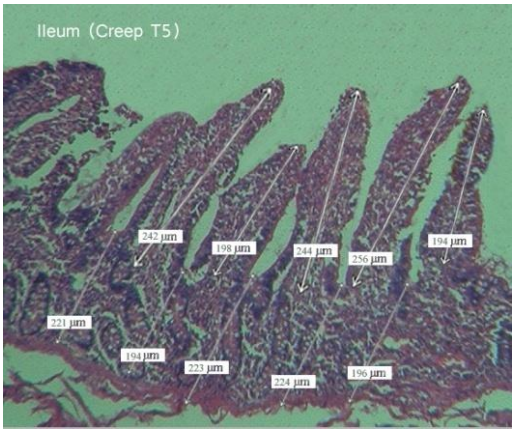
T2 (PC)



T3

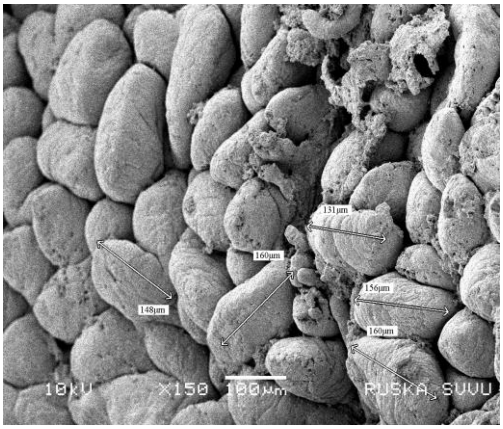


T4

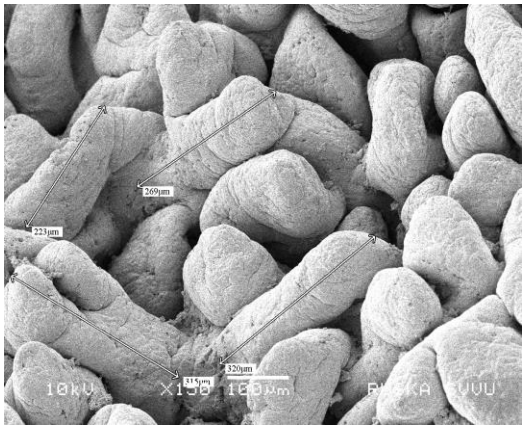


T5

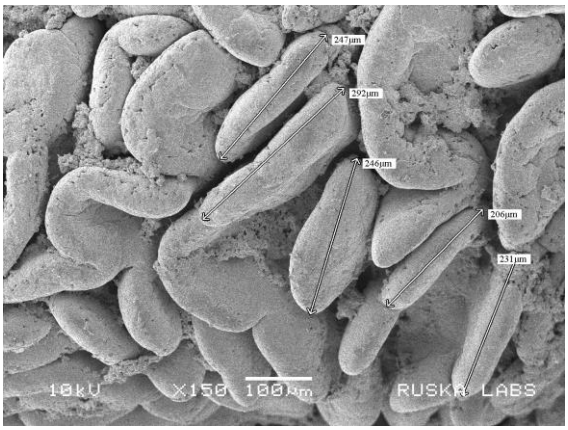
Fig.8: Electron-micrographs of small intestine (Ileum) of pre-weaned piglets showing villi height (SEM images)



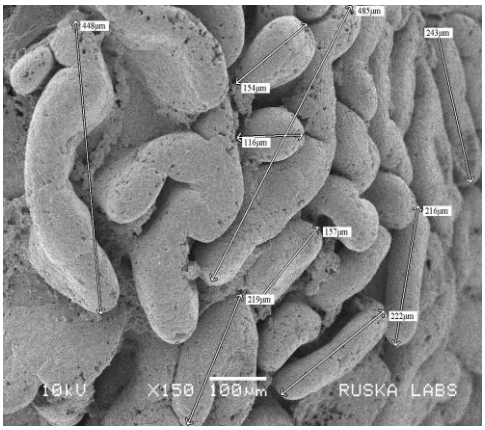
T1 (NC)



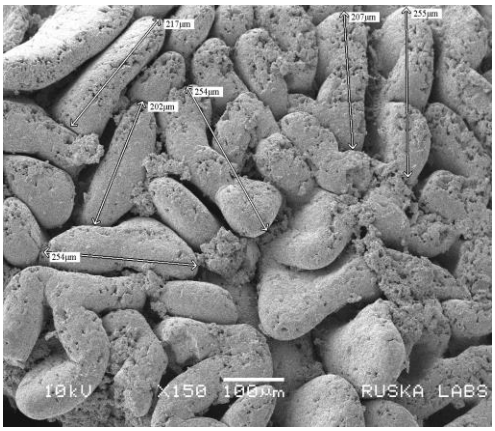
T2 (PC)



T3



T4



T5

4.5.2. In growers

The effect of different dietary treatments on small intestinal morphological feachers in growers is presented in Table 8 and Fig. 9-11. The height of villi in duodenum of pigs fed on T4 ration was significantly the highest (P<0.01), while the villus height of other treatments was in order of T3>T2>T5>T1 and the values were 174.8, 181.2, 181.8, 262.0 and 175.2 for T1 to T5 fed pigs, respectively.The crypt depth in duodenum of pigs fed on T4 was significantly the lowest (P<0.01) and the crypt depth of T2 and T5 fed pigs was lower than T1 and T3 fed pigs and the values were 176.8, 153.6, 176.0, 121.2 and 151.2 for T1 to T5 fed pigs, respectively. The ratio of villus height to crypt depth (VH/CD) in duodenum is highest (P<0.01) in pigs fed T4 ration and the ratio of villus height to crypt depth is higher in T2 and T5 when compared to T3 and T1 and the values were 0.98, 1.17, 1.03, 2.16 and 1.15 for T1 to T5 fed pigs, respectively.

The height of villi in jejunum of pigs fed T4 ration was significantly the highest (P<0.01) and the values were 178.8, 184.6, 183.2, 248.8 and 196.8 for T1 to T5 fed pigs, respectively.The crypt depth in jejunum of pigs fed on T4 ration was again significantly the lowest (P<0.01) and the values were 181.2, 164.4, 162.0, 129.8 and 181.6 for T1 to T5 fed pigs, respectively.The ratio of villus height to crypt depth (VH/CD) in jejunum was higher (P<0.01) in pigs fed T4 and the values were 0.98, 1.13, 1.13, 1.92 and 1.08 for T1 to T5 fed pigs, respectively.

The height of villi in ileum of pigs fed T4 ration was significantly the highest (P<0.01), while in T2 and T5 fed pigs it was higher than in T1 and T3 and the values were 177.2, 188.4, 179.6, 250.4 and 197.2 for T1 to T5 fed pigs, respectively. The crypt depth in ileum of pigs were significantly different (P<0.01) among the treatments and were in the order of T4>T5>T1>T2 and T3

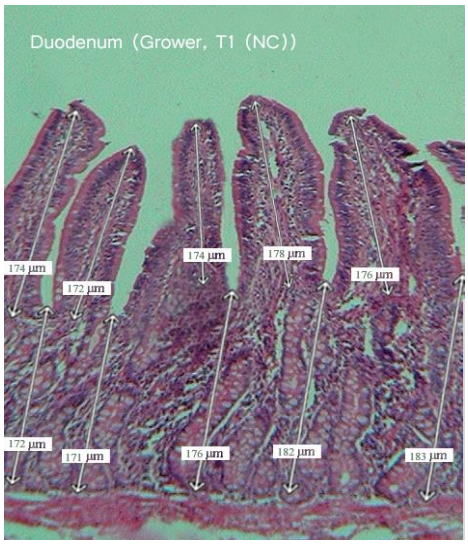
and the values were 177.8, 172.4, 168.0, 194.8 and 186.8 for T1 to T5 fed pigs, respectively. The ratio of villus height to crypt depth (VH/CD) in ileum is height ($P<0.01$) in pigs fed T4 ration. The ratio of villus height to crypt depth was in order of T2>T3>T5>T1 fed pigs and the difference was significant ($P<0.01$). The values were 0.99, 1.09, 1.06, 1.28 and 1.05 for T1 to T5 fed pigs, respectively.

Table 8: Effectof experimental dietson small intestinalmorphological structure in growers[1]

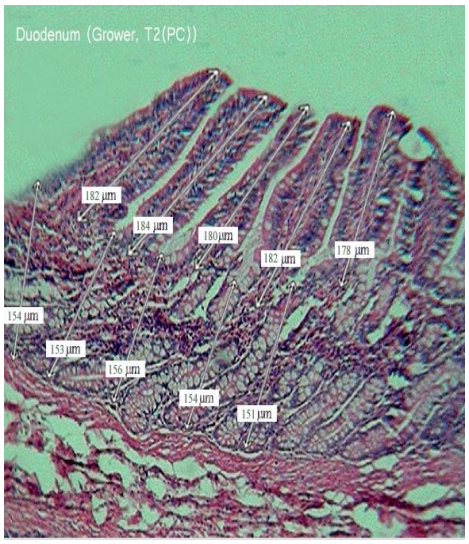
Item		Treatments				
		T1 (NC)	**T2 (PC)**	**T3**	**T4**	**T5**
Duodenum	Villus height (µm)	$174.8^{c}\pm1.02$	$181.2^{b}\pm1.02$	$181.8^{b}\pm0.8$	$262.0^{a}\pm2.60$	$175.2^{c}\pm2.87$
	Crypt depth (µm)	$176.8^{a}\pm2.47$	$153.6^{b}\pm0.81$	$176.0^{a}\pm2.19$	$121.2^{c}\pm2.41$	$151.2^{b}\pm1.02$
	VH/CD[2]	$0.98^{c}\pm0.01$	$1.17^{b}\pm0.01$	$1.03^{c}\pm0.01$	$2.16^{a}\pm0.04$	$1.15^{b}\pm0.01$
Jejunum	Villus height (µm)	$178.8^{d}\pm1.85$	$184.6^{c}\pm2.27$	$183.2^{cd}\pm1.49$	$248.8^{a}\pm1.85$	$196.8^{b}\pm1.35$
	Crypt depth (µm)	$181.2^{a}\pm1.02$	$164.4^{ab}\pm9.57$	$162.0^{b}\pm7.72$	$129.8^{c}\pm2.49$	$181.6^{a}\pm3.90$
	VH/CD[2]	$0.98^{c}\pm0.01$	$1.13^{b}\pm0.01$	$1.13^{b}\pm0.04$	$1.92^{a}\pm0.04$	$1.08^{bc}\pm0.01$
Ileum	Villus height (µm)	$177.2^{d}\pm1.35$	$188.4^{c}\pm2.13$	$179.6^{d}\pm1.93$	$250.4^{a}\pm4.16$	$197.2^{b}\pm1.02$
	Crypt depth (µm)	$177.8^{c}\pm1.62$	$172.4^{d}\pm0.74$	$168.0^{c}\pm1.41$	$194.8^{a}\pm1.35$	$186.8^{b}\pm1.49$
	VH/CD[2]	$0.99^{c}\pm0.01$	$1.09^{b}\pm0.01$	$1.06^{b}\pm0.02$	$1.28^{a}\pm0.02$	$1.05^{b}\pm0.01$

1 The number of observations for each mean value was five (n=5)
2 VH/CD means the ratio of villus height to crypt depth
^{abcd}values in a row not sharing common superscripts differ significantly (P<0.01)

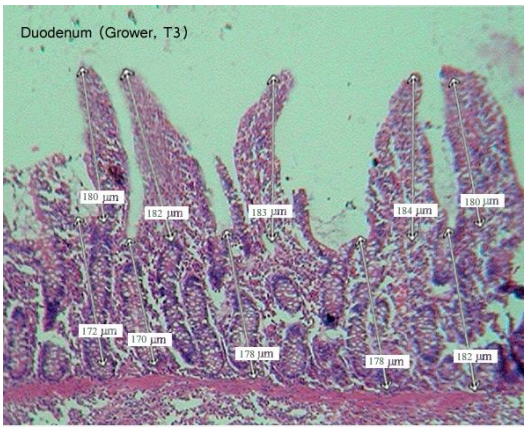
Fig.9 : Photomicrograph of small intestine (Duodenum) of growers showing villi height and cryptdepth (Hematoxylin and Eosine x 10X)



T1 (NC)



T2 (PC)



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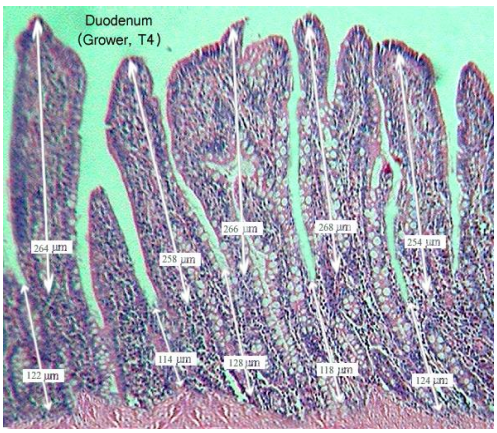
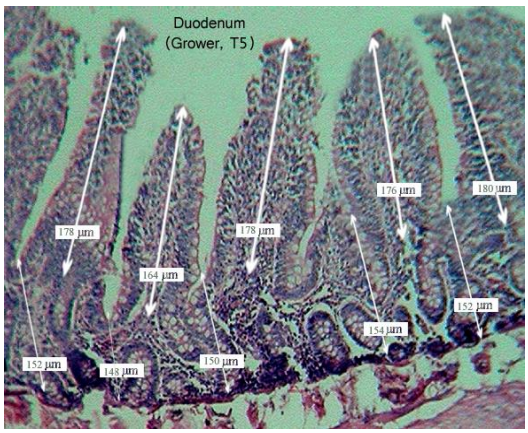
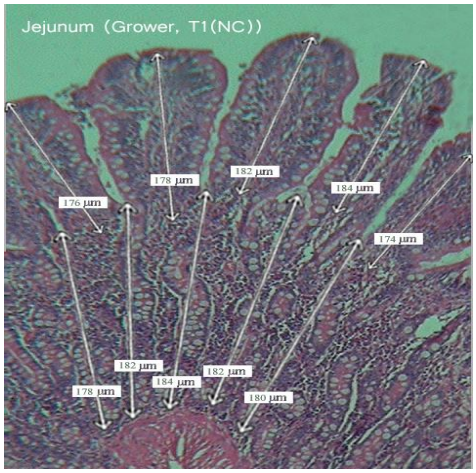


T4

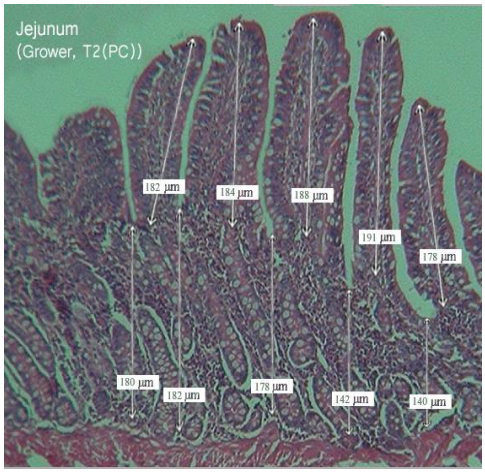


T5

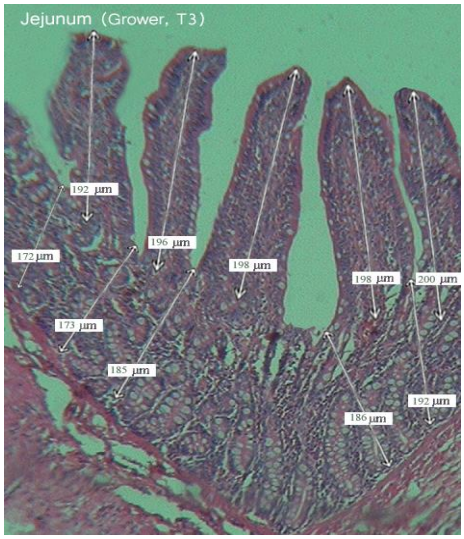
Fig.10: Photomicrograph of small intestine (Jejunum) of growers showing villi height and cryptdepth (Hematoxylin and Eosine x 10X)



T1 (NC)

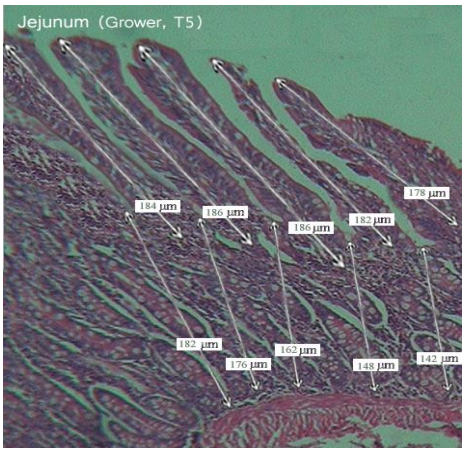


T2 (PC)



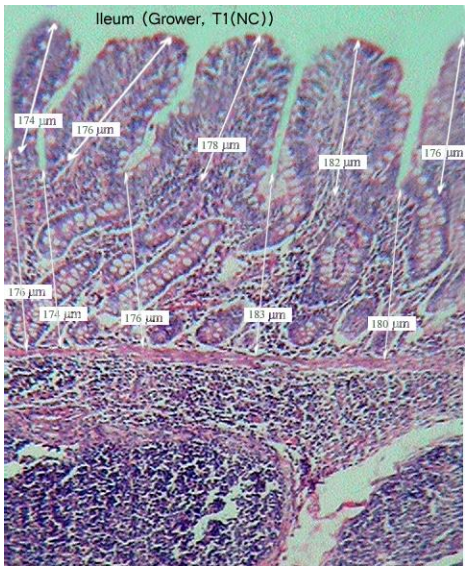
T3

T4

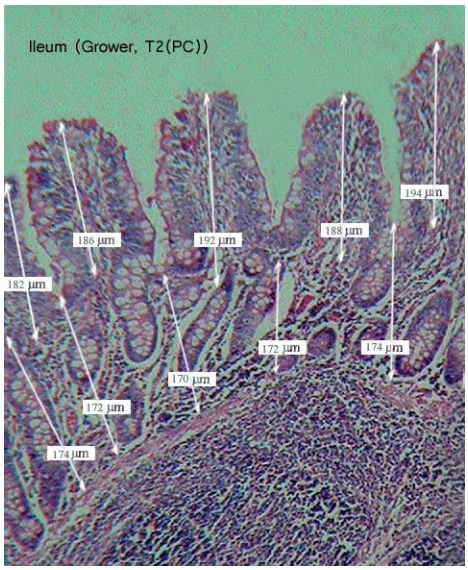


T5

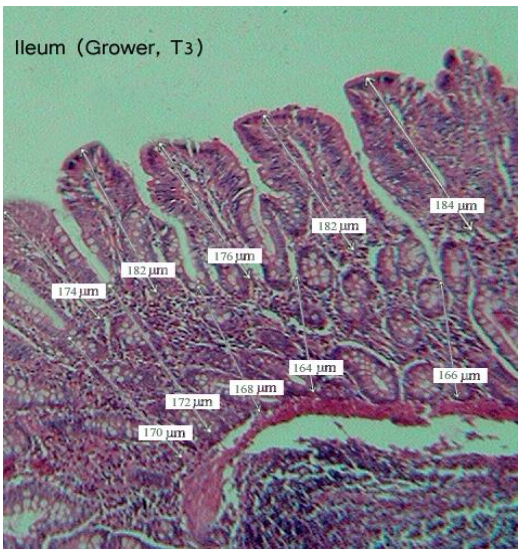
Fig.11: Photomicrograph of small intestine (Ileum) of growers showing villi height and cryptdepth (Hematoxylin and Eosine x 10X)



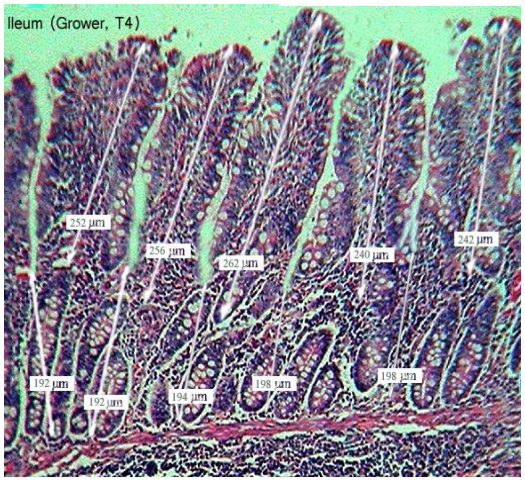
T1 (NC)



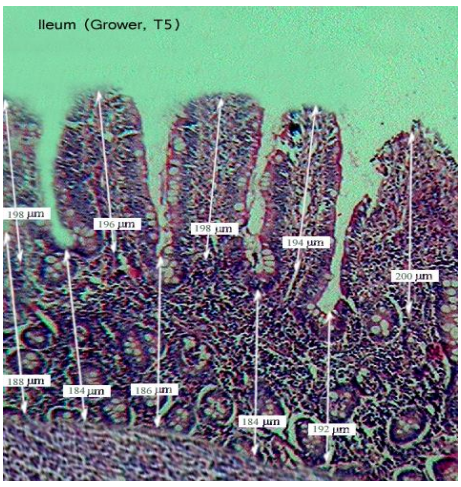
T2 (PC)



T3



T4



T5

4.5.3. In finishers

The effect of dietary treatments on small intestinal morphological feachers in finisher pigs is presented in Table 9 and Fig 12-14. The height of villi in duodenum of pigs fed on T4 ration was significantly higher ($P<0.01$), while in T2 and T5 fed pigs it was higher ($P<0.01$) than in T1 and T3 and the values were 177.4, 190.6, 176.8, 202.2 and 186.2 for T1 to T5 fed pigs, respectively. The crypt depth in duodenum of pigs fed on T4 was significantly lower ($P<0.01$) than in other treatments and crypt depth of T2 and T3 fed pigs was lower ($P<0.01$) than inT1 and T5 fed pigs and the values were 175.6, 164.0, 163.8, 73.6 and 169.4 for T1 to T5 fed pigs, respectively. The ratio of villus height to crypt depth (VH/CD) in duodenum was wider ($P<0.01$) in pigs fed T4 ration when compared to other treatments and the values were 0.99, 1.17, 1.07, 2.76 and 1.10 for T1 to T5 fed pigs, respectively.

The height of villi in jejunum of pigs fed T4 and T5 rations was significantly higher ($P<0.01$), while in T2 fed pigs it was higher than in T1 and T3 and the values were 176.4, 182.8, 178.4, 187.2 and 188.4 for T1 to T5 fed pigs, respectively. The crypt depth in jejunum of pigs fed on T4 ration was significantly lower ($P<0.01$) than in other treatments. The crypt depth of jejunum of pigs fed on T2 ration was significantly lower than crypt depth of jejunum of pigs fed on T1, T3 and T5 rations. The values for crypt depth of jejunum were 176.0, 169.2, 173.4, 96.8 and 177.2 for T1 to T5 fed pigs, respectively. The ratio of villus height to crypt depth (VH/CD) in jejunum was highest ($P<0.01$) in pigs fed T4 ration and villus height to crypt depth ratio was significantly higher ($P<0.01$) in T2, T3 and T5 fed pigs than T1 and the values were 0.99, 1.08, 1.02, 1.93 and 1.06 for T1 to T5 fed pigs, respectively.

The height of villi in ileum of pigs fed T4 ration was significantly higher ($P<0.01$), while in T2 and T5 fed pigs it was higher than in T1 and T3 and the values were 172.0, 182.0, 171.4, 204.0 and 180.8 for T1 to T5 fed pigs, respectively. The crypt depth in ileum of pigs fed T4 was lowest ($P<0.01$), while the crypt depth of ileum of pigs fed T2 was lower than in T3 and T4. The crypt depths of ileum for T1 to T5 were 175.8, 154.0, 168.2, 88.4 and 164.0, respectively. The ratio of villus height to crypt depth (VH/CD) in ileum was height ($P<0.01$) in pigs fed T4 ration. The ratio of villus height to crypt depth was in order of T2>T5> T3>T1 fed pigs and the differences were significant ($P<0.01$). The values were 0.97, 1.18, 1.01, 2.30 and 1.10 for T1 to T5 fed pigs, respectively.

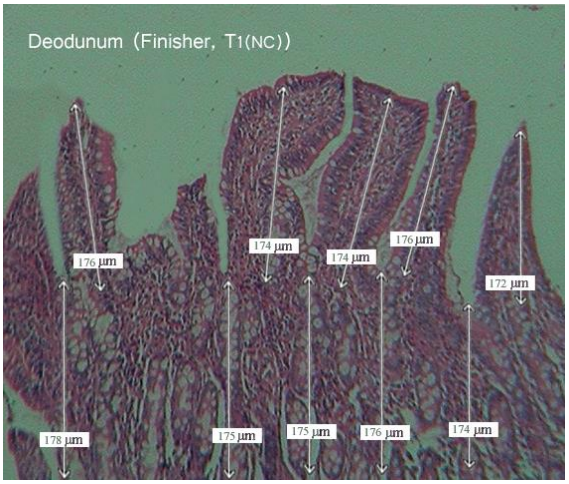
Table 9: Effectof experimental dietson small intestinalmorphological structure in finishers[1]

Item		Treatments				
		T1 (NC)	**T2 (PC)**	**T3**	**T4**	**T5**
Duodenum	Villus height (µm)	$174.4^{c}\pm0.74$	$190.6^{b}\pm1.32$	$176.8^{c}\pm1.02$	$202.2^{a}\pm5.46$	$186.2^{b}\pm1.12$
	Crypt depth (µm)	$175.6^{a}\pm0.67$	$164.0^{b}\pm6.96$	$163.8^{b}\pm2.8$	$73.6^{c}\pm1.46$	$169.4^{ab}\pm2.04$
	VH/CD[2]	$0.99^{b}\pm0.01$	$1.17^{b}\pm0.05$	$1.07^{b}\pm0.01$	$2.76^{a}\pm0.12$	$1.1^{b}\pm0.01$
Jejunum	Villus height (µm)	$176.4^{c}\pm1.16$	$182.8^{b}\pm1.02$	$178.4^{c}\pm1.91$	$187.2^{a}\pm1.62$	$188.4^{a}\pm0.97$
	Crypt depth (µm)	$176.0^{a}\pm1.67$	$169.2^{b}\pm3.32$	$173.4^{ab}\pm1.88$	$96.8^{c}\pm1.82$	$177.2^{a}\pm2.33$
	VH/CD[2]	$0.99^{c}\pm0.01$	$1.08^{b}\pm0.02$	$1.02^{bc}\pm0.02$	$1.93^{a}\pm0.02$	$1.06^{bc}\pm0.02$
Ileum	Villus height (µm)	$172.0^{c}\pm1.89$	$182.0^{b}\pm2.09$	$171.4^{c}\pm2.31$	$204.0^{a}\pm0.70$	$180.8^{b}\pm1.35$
	Crypt depth (µm)	$175.8^{a}\pm1.28$	$154.0^{c}\pm1.41$	$168.2^{b}\pm1.90$	$88.4^{d}\pm1.02$	$164.0^{b}\pm3.16$
	VH/CD[2]	$0.97^{d}\pm0.01$	$1.18^{b}\pm0.01$	$1.01^{d}\pm0.01$	$2.30^{a}\pm0.03$	$1.10^{c}\pm0.02$

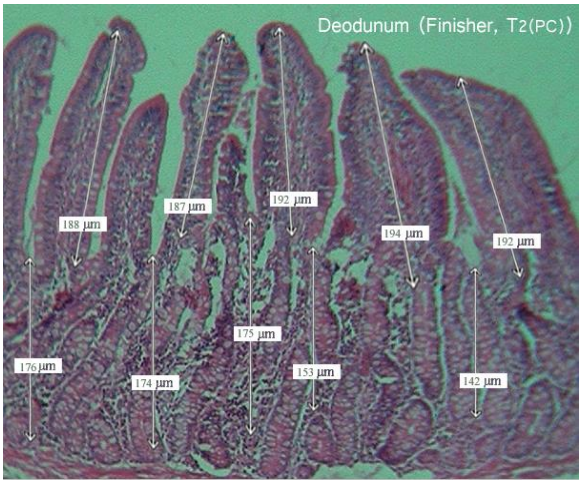
1 The number of observations for each mean value was five (n=5)
2 VH/CD means the ratio of villus height to crypt depth

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

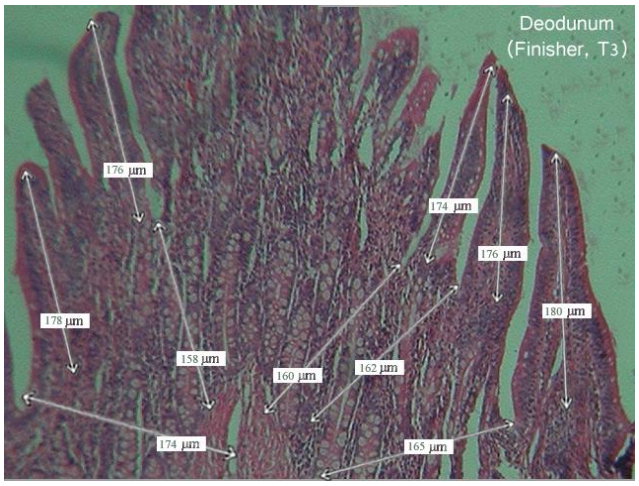
Fig.12: Photomicrograph of small intestine (Duodenum) of finishers showing villi height and cryptdepth (Hematoxylin and Eosine x 10X)



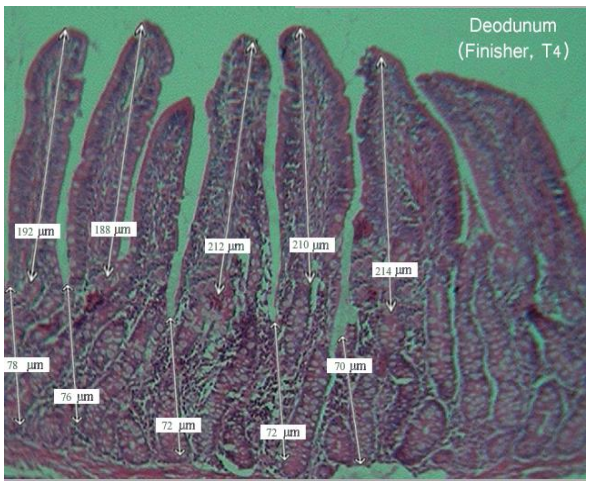
T1 (NC)



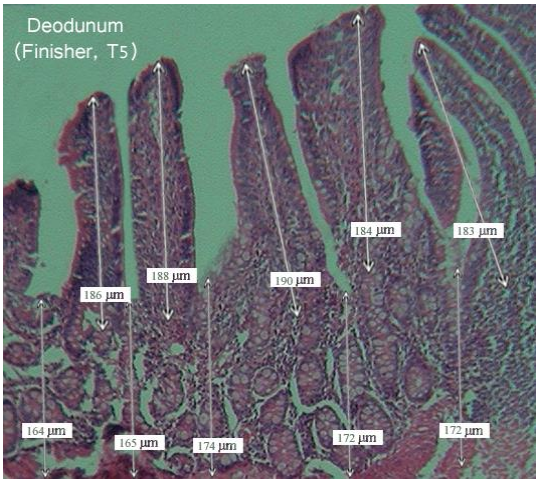
T2 (PC)



T3

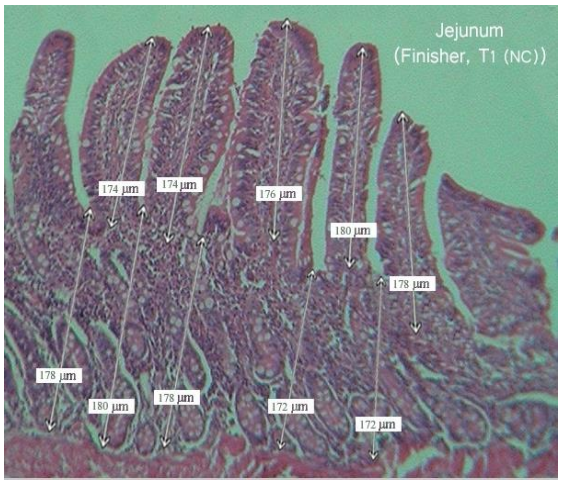


T4

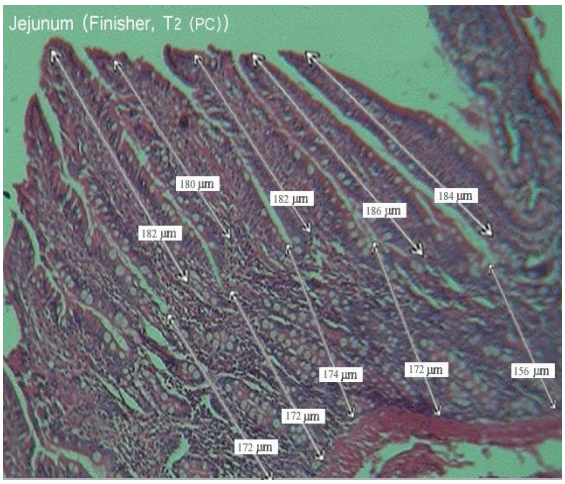


T5

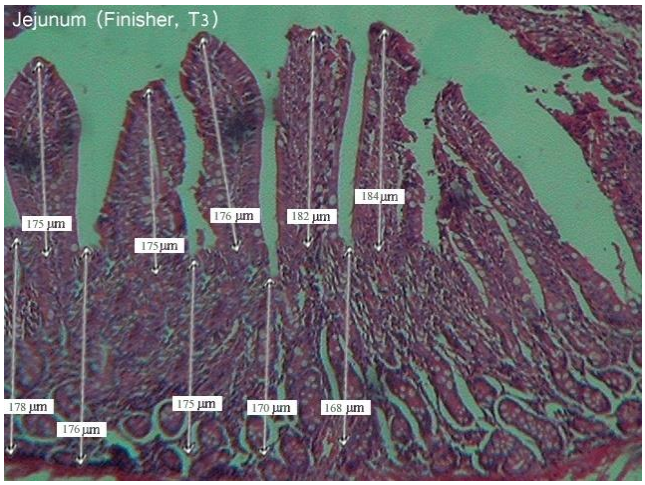
Fig.13: Photomicrograph of small intestine (Jejunum) of finishers showing villi height and cryptdepth (Hematoxylin and Eosine x 10X)



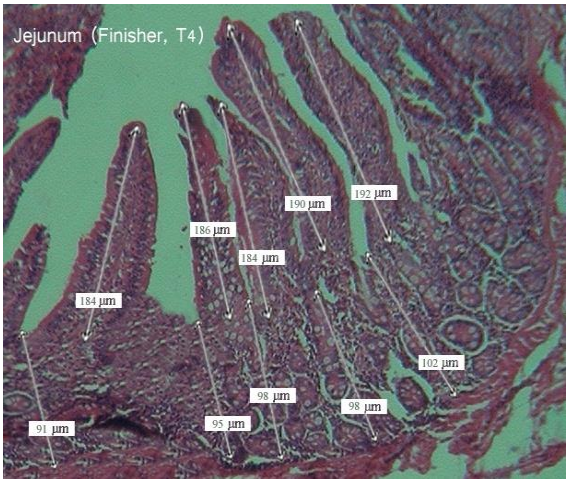
T1 (NC)



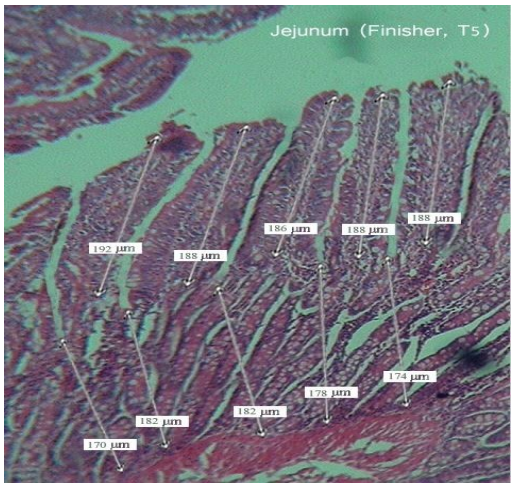
T2 (PC)



T3

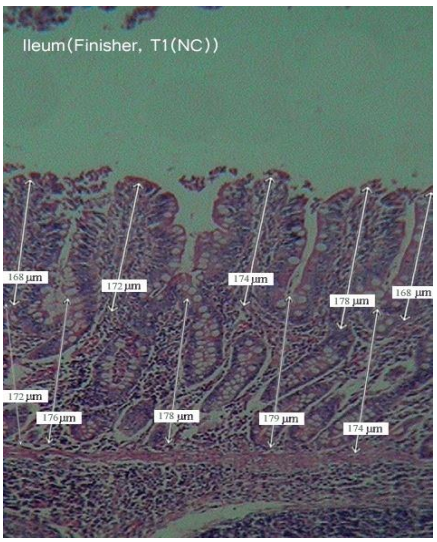


T4

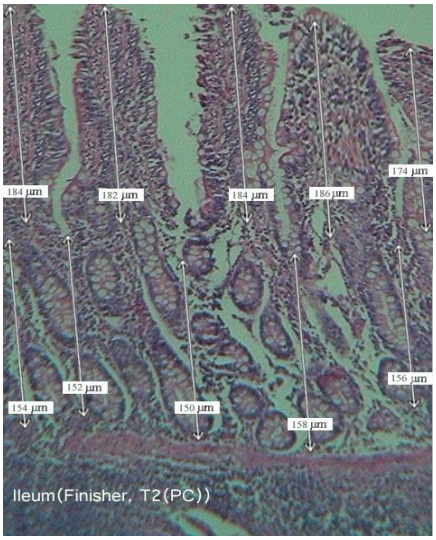


T5

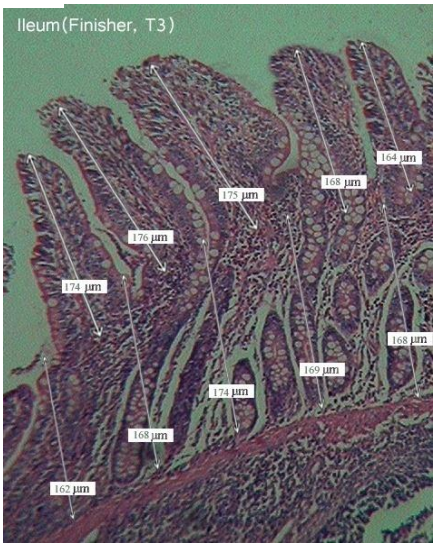
Fig.14: Photomicrograph of small intestine (Ileum) of finishers showing villi height and cryptdepth (Hematoxylin and Eosine x 10X)



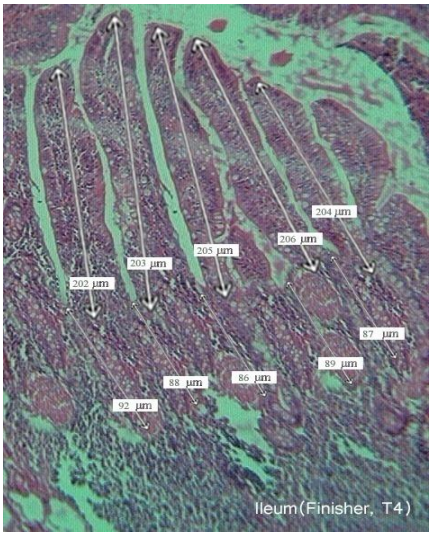
T1 (NC)



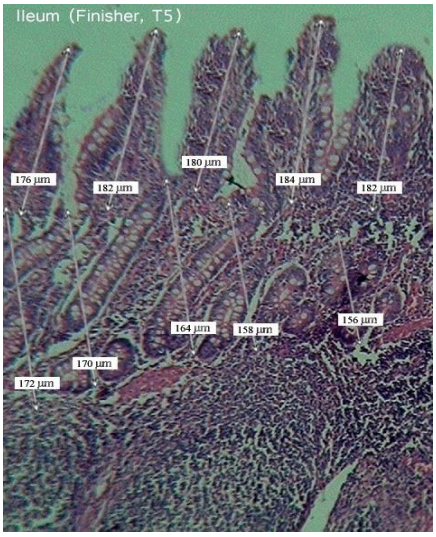
T2 (PC)



T3



T4



T5

4.6. Effect of dietary treatments on pathogenic bacteria of largeintestinal contents

The effect of experimental diets on pathogenic bacteria of large intestinal contents is presented in Table 10 and Fig.15. Feeding diets containing antibiotic (T2) and shrimp shell meal containing chitosan (T3 to T5) reduced ($P<0.01$) the *E. Coli* and *Salmonella* count when compared to control group and the values (cfu/gm) were 72.36, 25.81, 35.70, 26.77 and 18.37 (*E.coli*); 61.25, 21.49, 34.61, 22.65 and 14.17 (*Salmonella*) in pigs fed T1 to T5, respectively. The pathogenic bacterial count of large intestinal contents of crossbred pigs at the end of creep and grower phases was also studied and presented in Table 11 and 12 and Figs16 and 17. During creep phase the *E. coli* as well as *Salmonella* count (cfu/gm) was reduced in diets containing antibiotics (T2) and shrimp shell meal (T3 to T5) and the values were 76.35, 24.09, 32.23, 23.67 and 19.37 (*E.coli*); 53.52, 31.13, 40.29, 29.98 and 16.99 (*Salmonella)* for fed T1 to T5, respectively. The same trend was also noticed at the end of grower phase and the values were 70.75, 18.81, 27.49, 16.47 and 11.40 (*E.coli*); 60.01, 24.31, 37.51, 24.30 and 18.33 (*Salmonella*)inpigs fed T1toT5, respectively.

Table 10: Effect of dietary treatments on pathogenic bacteria (cfu/g) of large intestinal content in preweaned pigs

Pathogenic bacteria	T1	T2	T3	T4	T5
*E.coli***	$76.35^{a}\pm2.83$	$24.09^{c}\pm0.52$	$32.23^{b}\pm1.17$	$23.67^{c}\pm1.04$	$19.37^{d}\pm0.62$
*Salmonella***	$53.52^{a}\pm0.86$	$31.13^{c}\pm0.62$	$40.29^{b}\pm0.05$	$29.98^{c}\pm0.13$	$16.99^{d}\pm1.33$

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

Table 11: Effect of dietary treatments on pathogenic bacteria (cfu/g) of large intestinal content in growing pigs

Pathogenic bacteria	T1	T2	T3	T4	T5
*E.coli***	$70.75^{a}\pm1.57$	$18.8^{c}\pm0.19$	$27.49^{b}\pm1.16$	$16.47^{c}\pm1.92$	$11.40^{d}\pm1.22$
*Salmonella***	$60.01^{a}\pm1.68$	$24.31^{c}\pm0.60$	$37.51^{b}\pm1.40$	$24.30^{c}\pm1.67$	$18.33^{c}\pm2.34$

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

Table 12: Effect of dietary treatments on pathogenic bacteria (cfu/g) of large intestinal content in finishing pigs

Pathogenic bacteria	T1	T2	T3	T4	T5
*E.coli***	$72.36^{a}\pm1.30$	$25.81^{c}\pm0.50$	$35.70^{b}\pm0.45$	$26.77^{c}\pm0.56$	$18.37^{d}\pm0.35$
*Salmonella***	$61.25^{a}\pm0.73$	$21.49^{c}\pm0.57$	$34.61^{b}\pm0.22$	$22.65^{c}\pm0.38$	$14.17^{d}\pm0.18$

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

Fig.15: Effect of dietary treatments on pathogenic bacteria (cfu/g) of large intestinal contents in preweaned pigs

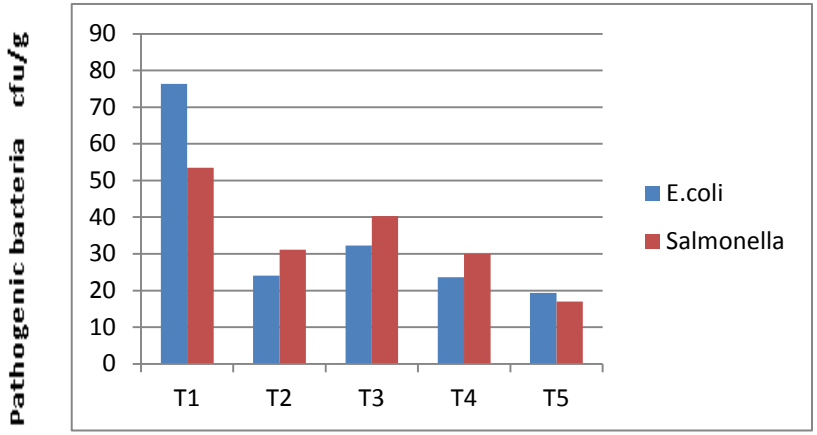


Fig.16: Effect of dietary treatments on pathogenic bacteria (cfu/g) of large intestinal contents in growing pigs

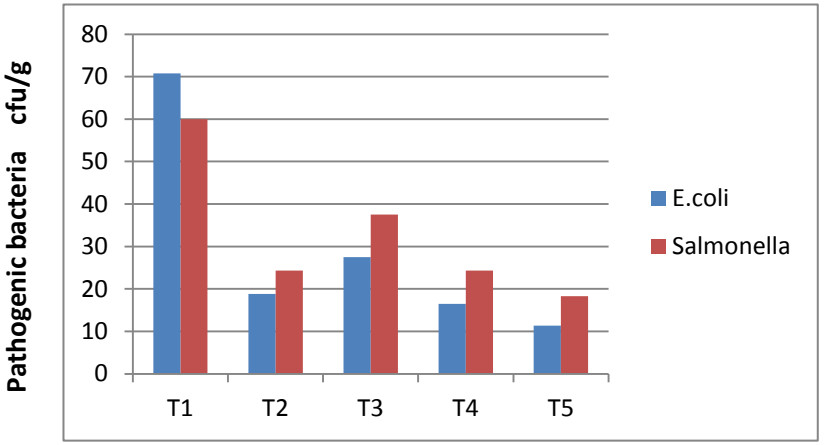
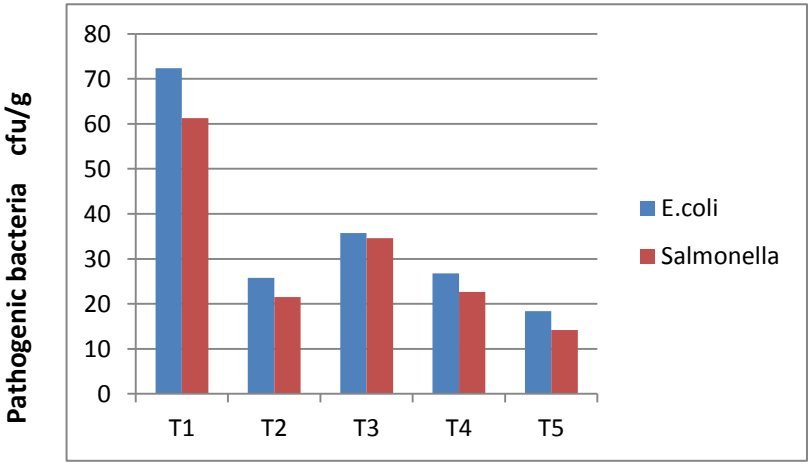


Fig.17: Effect of dietary treatments on pathogenic bacteria (cfu/g)of large intestinal contents in finishing pigs



4.7. Metabolism trial in finishing pigs

4.7.1. Digestibility of nutrients

The digestibility (%) of nutrients is presented in Table 13. The digestibility of DM was higher (P<0.05) in pigs fed T4 and T5 rations than in T3 and the values were 85.27, 85.84, 84.08, 87.69 and 86.61 in pigs fed T1 to T5, respectively. The digestibility of CPin pigs fed T4 was significantly higher (P<0.01) than in other treatments and the values were 85.00, 85.98, 85.13, 89.65 and 85.01 % for T1 to T5 fed pigs, respectively. The CF digestibility was significantly higher (P<0.01) in T4 and T5 fed pigs compared to T1, T2 and T3 and the values were 53.79, 56.78, 56.80, 64.75 and 64.85 % for T1 to T5 fed pigs, respectively. The digestibility of EE was significantly higher (P<0.01) in T1 and T2 than in other treatments. The digestibility of NFE was significantly higher (P<0.05) in T4 and T5 when compared to T1, T2 and T3 and the values were 90.37, 90.91, 90.76, 93.22 and 93.15 % for T1 to T5 fed pigs, respectively.

Table13: Effect of dietary treatments on nutrient digestibility (%) during finisher phase

Digestibility (%)	T1	T2	T3	T4	T5
DM*	85.27^{ab}±0.58	85.84^{ab}±0.86	84.08^{b}±0.88	87.69^{a}±1.00	86.61^{a}±0.82
CP**	85.00^{b}±0.61	85.98^{b}±0.50	85.13^{b}±0.58	89.65^{a}±0.81	85.01^{b}±1.06
CF**	53.79^{b}±0.51	56.78^{b}±1.61	56.80^{b}±2.10	64.75^{a}±1.40	64.85^{a}±1.25
EE**	79.99^{a}±1.31	79.49^{a}±1.67	73.01^{b}±1.49	71.15^{b}±0.59	69.80^{b}±2.20
NFE*	90.37^{b}±0.59	90.91^{b}±0.76	90.76^{b}±0.87	93.22^{a}±0.81	93.15^{a}±0.74

^{ab}values in a row not sharing common superscripts differ significantly *($P<0.05$)

4.7.2. Nitrogen balance

Nitrogen balance of pigs fed experimental diets during finisher phase is presented in Table 14. The nitrogen intake (g/d) was higher (P<0.01)in T1 (51.18) than in pigs fed T4 (48.80), T5 (50.05), T2 (50.61) or T3 (50.69). The nitrogen loss in faeces (g/d) was lower (P<0.01) in T4 than in other treatments and the values were 7.82, 7.09, 7.65 and 7.33 for T1, T2,T3 and T5, respectively. The nitrogen loss in urine (g/d) was higher (P<0.05) in T1 (9.12) and T4 (8.41) than in T2 (6.54) where as in T3 (7.67) and T5(7.69) it was not significantly different from other treatments. The total nitrogen loss (g/d) was in the order of T1 (16.92) > T5 (15.03) > T3 (14.82) > T2 (13.63) >T4 (13.46) fed pigs and the differences were significant (P<0.01). The nitrogen retained (g/d) was higher (P<0.05) in T2 (36.92), followed by T3 (35.36), T4 (35.34), T5 (35.02) or T1 (34.27) fed pigs. The nitrogen retention as (% of intake) was higher (P<0.01) in T4 (72.33), followed by T2 (71.61), T5 (69.97), T3 (69.32), or T1 (66.93) fed pigs. The nitrogen retention as (% of absorbed) was comparable among the treatments and the values were 78.97, 80.99, 80.33, 80.83 and 80.07 for T1 to T5 fed pigs, respectively.

Table 14: Effect of dietary treatments on nitrogen balance during finisher phase

Nitrogen balance	T1	T2	T3	T4	T5
Intake (g/d) **	51.18^{a}±0.02	50.61^{ab}±0.06	50.69^{ab}±0.21	48.80^{c}±0.61	50.05^{b}±0.19
Outgo in faeces (g/d)**	7.82^{a}±0.34	7.09^{a}±0.24	7.65^{a}±0.37	5.04^{b}±0.39	7.33^{a}±0.30
Outgo in urine (g/d)*	9.12^{a}±0.43	6.54^{c}±0.61	7.67^{abc}±0.34	8.41^{b}±0.53	7.69^{abc}±0.55
Total loss (g/d)**	16.92^{a}±0.45	13.63^{b}±0.49	14.82^{b}±0.73	13.46^{b}±0.55	15.03^{b}±0.42
Retained (g/d)*	34.27^{c}±0.45	36.92^{a}±0.49	35.36^{abc}±0.38	35.34^{abc}±1.12	35.02^{b}±0.32
Retained (% of intake)**	66.93^{d}±0.89	71.61^{ab}±0.43	69.32^{bcd}±0.73	72.33^{a}±1.46	69.97^{bc}±0.76
Retained (% of absorbed)^{NS}	78.97±0.96	80.99±0.29	80.33±0.49	80.83±0.65	80.07±1.05

^{abcd}values in a row not sharing common superscripts differ significantly *(P<0.05) **(P<0.01)

^{NS}Notsignificant

4.8 Growth performance

4.8.1. Creep phase (10 to 42 days)

The growth performance of the piglets fed creep diets is presented in Table 15. The initial litter weight (kg) was not significantly different among the treatments and the values were 11.31(T1), 11.91(T2), 11.23(T3), 11.51(T4) and 11.2(T5) whereas at the end of creep phase (42 days) it was significantly higher ($P<0.01$) in T2 to T5 than in T1 fed pigs and the values were 66.01(T1), 73.33(T2), 75.16(T3), 75.83(T4) and 79.56 kg (T5). The total weight gain (kg) of litter was lower ($P<0.05$) in T1 than in other treatments and the values were 54.73, 61.41, 63.93,64.33 and 70.13 in piglets fed T1 to T5, respectively. There was no significant difference among different treatments intotal creepfeed intake (kg) and the values were46.83, 48.18, 47.0, 45.83 and 49.08 for T1 to T5 fed piglets, respectively. The feed per kg gain was higher ($P<0.05$) in T1 than in other treatments and the values were 0.88, 0.78, 0.73, 0.65 and 0.71 for T1 to T5 fed piglets, respectively. The cost of feed per kg gain was lower($P<0.05$) in T4 than in other treatments and the values were (Rs.) 19.0, 17.2, 16.17, 14.55 and 15.87 in T1 to T5 fed piglets, respectively.

Table 15: Effect of dietary treatments on growth performance of crossbred pigs during creep phase (upto 42 days)

Parameter	T1	T2	T3	T4	T5
Initial weight (kg) ^{NS}	11.31±0.58	11.91±0.59	11.23±0.35	11.51±0.36	11.20±0.36
Final weight (kg)*	66.01^{b}±2.18	73.33^{a}±4.38	75.16^{a}±0.79	75.83^{a}±1.54	79.56^{a}±3.08
Weight gain (kg) **	54.73^{c}±1.94	61.41^{bc}±3.85	63.93^{ab}±0.72	64.33^{ab}±1.38	70.13^{a}±2.29
No. of days	42	42	42	42	42
Total feed intake(kg) ^{NS}	46.83±1.22	48.18±2.43	47.0±4.91	45.83±4.85	49.08±4.11
Feed / kg gain**	0.88^{a}±0.03	0.78^{b}±0.03	0.73^{b}±0.02	0.65^{c}±0.02	0.71^{bc}±0.02
Cost of feed/kg gain** (Rs.)	19.0^{a}±0.77	17.2^{b}± 0.51	16.17^{b}± 0.26	14.55^{c} ±0.43	15.87^{bc}± 0.37

^{abcd}values in a row not sharing common superscripts differ significantly *(P<0.05) **(P<0.01)^{NS} Not significant

4.8.2. Grower phase (15 kg to 35 kg body weight)

The growth performance of the pigs fed grower diets is presented in Table 16. The initial and final weights (kg) were not significantly different among treatments and the values were 15.77, 35.12 (T1), 15.76, 35.12 (T2), 15.73, 35.25 (T3), 15.78, 35.25 (T4) and 15.75, 35.81 (T5). Similarly the weight gain (kg) was also not significantly different among treatments and the values were 19.35, 19.36, 19.51, 19.46 and 20.06 for T1 to T5 fed pigs,, respectively. However, the pigs fed T4 had taken lower ($P<0.01$) number of days (55) than those fed T1 (72), while in T5 it was comparable with other treatments and the values were 72, 63, 62, 55 and 61 days, respectively, for T1 to T5 fed pigs. The ADG (g) was higher ($P<0.01$) in T4(348) or T5 (324) fed pigs than in T3 (310),T2 (303) and T1 (267) fed pigs. The ADFI (kg) was also higher ($P<0.01$) in T5 (0.80) or T4 (0.78) fed pigs than in T1 (0.73), T2 (0.75) and T3 (0.75) fed pigs. The feed per kg gain and the cost of feed per kg gain (Rs) were higher ($P<0.01$) in T1 fed pigs than in other treatments and the values were 2.77 and 61.24 (T1), 2.47 and 54.47 (T2), 2.42 and 53.46(T3), 2.26 and 49.84 (T4) and 2.46 and 54.40 (T5) fed pigs, respectively.

Table 16: Effect of dietary treatments on growth performance of crossbred pigs during grower phase (15 to 35 kg body weight)

Parameter	T1	T2	T3	T4	T5
Initial weight (kg)^{NS}	15.77±0.23	15.76±0.18	15.73±0.28	15.78±0.16	15.75±0.20
Final weight (kg) ^{NS}	35.12±0.65	35.12±0.29	35.25±0.67	35.25±0.72	35.81±0.36
Weight gain (kg) ^{NS}	19.35±0.69	19.36±0.23	19.51±0.58	19.46±0.65	20.06±0.35
No. of days**	72.25^{a}±0.67	63.75^{b}±0.36	62.75^{bc}±0.36	55.87^{d}±0.71	61.75^{c}±0.25
ADG (g)**	267.24^{d}±7.40	303.76^{c}±3.62	310.70^{bc}±7.82	348.24^{a}±0.58	324.84^{b}±5.21
ADFI (kg)**	0.73^{b}±0.01	0.75^{b}±0.01	0.75^{b}±0.01	0.78^{a}±0.01	0.80^{a}±0.01
Feed / kg gain**	2.77^{a}±0.11	2.47^{b}±0.04	2.42^{b}±0.06	2.26^{b}±0.08	2.46^{b}±0.04
Cost of feed/kg gain (Rs.)**	61.24^{a}±2.46	54.47^{b}±0.98	53.46^{b}±1.37	49.84^{b}±1.82	54.40^{b}±0.91

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

^{NS} Not significant

4.8.3. Finisher phase (35 kg to 70 kg body weight)

The growth performance of pigs during finisher stage is presented in Table 17. The initial and final weights and total weight gain (kg) were not significantly different among the treatments and the values were 35.50, 70.75, 35.25; 35.08, 70.66, 35.58;35.91, 71.33, 35.41; 35.75, 70.91, 35.16 and 35.75, 71.41, 35.66, respectively for T1 to T5 fed pigs. The number of days taken was lower ($P<0.01$) in T4 (72) or T5 (79) than in T2 (90), T3 (80) or T1 (104) fed pigs. The ADG (g) was higher ($P<0.01$) in T4 (485) followed by T5 (451) than in T1 (338), T2 (393) or T3 (439) fed pigs. The ADFI (kg) was not significantly different among treatments and the values were 2.13, 2.12, 2.05, 2.03 and 2.05 in T1 to T5, respectively. The feed intake per kg gain (kg) was highest ($P<0.01$) in T1 (6.28) than in T2 (5.39), T3 (4.67), T4 (4.19) or T5 (4.55), fed pigs. The cost of feed per kg gain (Rs.) was significantly lower ($P<0.01$) in T4 (92.43), T5 (100.34), T3 (102.98) than in T2 (118.89) and T1(138.65) fed pigs, respectively.

Table 17: Effect of dietary treatments on growth performance of crossbred pigs during finisher phase (35 to 70 kg body weight)

Parameter	T1	T2	T3	T4	T5
Initial weight (kg)^{NS}	35.50±0.81	35.08±0.30	35.91±0.70	35.75±0.88	035.75±0.30
Final weight (kg) ^{NS}	70.75±0.25	70.66±0.42	71.33±0.33	70.91±0.30	71.41±0.20
Weight gain (kg) ^{NS}	35.25±0.97	35.58±0.49	35.41±0.71	35.16±0.99	35.66±0.40
No. of days**	104.16^{a}±1.53	90.66^{b}±1.40	80.66^{c}±1.47	72.50^{d}±1.11	79.16^{c}±1.53
ADG (g)**	338.16^{d}±5.70	393.20^{c}±10.43	439.60^{b}±10.72	485.00^{a}±10.89	451.02^{b}±6.34
ADFI (kg) ^{NS}	2.13±0.05	2.12±0.04	2.05±0.03	2.03±0.06	2.05±0.03
Feed / kg gain**	6.28^{a}±0.15	5.39^{b}±0.10	4.67^{c}±0.07	4.19^{d}±0.98	4.55^{c}±0.10
Cost of feed/kg gain (Rs.)**	138.65^{a}±3.51	118.89^{b}±2.20	102.98^{c}±1.68	92.43^{d}±2.03	100.34^{c}±2.23

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

^{NS} Not significant

4.8.4. Overall growth performance (15 kg to 70kg body weight)

The overall growth performance of pigs from grower to finisher stages is presented in Table 18. The initial, final and total weight gain (kg) were not significantly different among treatments and the values were 15.65, 71.25, 55.60; 15.85, 70.91, 55.06; 15.81, 71.33, 55.51; 15.81, 72.25, 56.43 and 15.65, 71.41, 55.76 in pigs fed T1 to T5, respectively. But the number of days taken to reach target weight was lower ($P<0.01$) in T4 (128) followed by T5 (140), T3 (143), T2 (154) fed pigs than in T1 (176).And also, the ADG (g) was higher ($P<0.01$) in T4 (438) followed by T5 (396) than in T3 (386), T2 (357) or T1 (314) fed pigs. The ADFI (kg) was not significantly different among treatments and the values were 1.55, 1.55, 1.47, 1.48 and 1.50 in pigs fed T1 to T5, respectively. The feed / kg gain was lower ($P<0.01$) in T4 (3.39) fed pigs than those fed T1 (4.94), T2 (4.34), T3 (3.81) or T5 (3.79). The cost of feed per kg gain (Rs.) was significantly lower ($P<0.01$) in pigs fed T4 (74.80) than in T1 (108.94), T2 (95.78), T3 (84.14) or T5 (83.64).

Table 18: Effect of dietary treatments on overall growth performance of crossbred pigs (15 to 70 kg body weight)

Parameter	T1	T2	T3	T4	T5
Initial weight (kg)^{NS}	15.65±0.24	15.85±0.22	15.81±0.32	15.81±0.17	15.65±0.24
Final weight (kg)*	71.25^{b}±0.25	70.91^{b}±0.41	71.33^{b}±0.33	72.25^{a}±0.17	71.41^{ab}±0.20
Weight gain (kg) ^{NS}	55.60±0.33	55.06±0.49	55.51±0.55	56.43±0.31	55.76±0.29
No. of days**	176.83^{a}±0.75	154.33^{b}±1.15	143.66^{c}±1.66	128.66^{d}±0.80	140.83^{c}±1.51
ADG (g)**	314.42^{d}±1.37	357.07^{c}±5.55	386.52^{b}±2.97	438.65^{a}±2.74	396.14^{b}±3.53
ADFI (kg) ^{NS}	1.55±0.04	1.55±0.02	1.47±0.02	1.48±0.04	1.50±0.02
Feed / kg gain**	4.94^{a}±0.12	4.34^{b}±0.05	3.81^{c}±0.04	3.39^{d}±0.09	3.79^{c}±0.07
Cost of feed/kg gain (Rs.)**	108.94^{a}±2.73	95.78^{b}±1.27	84.14^{c}±0.89	74.80^{d}±2.19	83.64^{c}±1.70

^{abcd}values in a row not sharing common superscripts differ significantly *(P<0.05) **(P<0.01)

^{NS} Not significant

4.9. Effect of dietary treatments on haematological characteristics

The results of the current study are shown in Table 21.There was significant increase (P<0.01) in PCV (%) and Hb (g/dl) in finisher pigs fed rations containing shrimp shell meal and antibiotics when compared to control group and the values were 31.78, 34.51, 34.88, 34.91and 32.94 (PCV); 10.73, 11.94, 12.27, 13.22 and 12.72 (Hb), respectively for T1 to T5 fed pigs. The RBC count was significantly higher (P<0.01) in T2 to T5 fed pigs when compared to T1 and the values were 6.10, 7.60, 7.38, 8.25 and 8.15 ($x10^6$/µl), respectively for T1 to T5 fed pigs. There was no significant difference in WBC count among treatments and the values were 15.54, 16.25, 16.25, 16.62 and 16.26 ($x10^3$/µl), respectively for T1 to T5 fed pigs, but neutrophils (%) and lymphocytes (%) were higher in T4 and T5 fed pigs when compared to other treatments and the values were 48.16, 43.16; 46.66, 45.50; 46.00, 45.00; 49.00, 47.16 and 50.33, 45.66, respectively for T1 to T5 fed pigs. There was no significant difference in basophils and the monocyte and eosinophil counts were significantly lower (P<0.01) in T4 and T5 fed pigs and the values were 4.83, 3.66; 5.00, 2.83; 5.66, 3.33; 2.83, 1.00 and 3.16, 0.83, respectively for T1 to T5 fed pigs.

The total protein (g/dl) content was highest (P<0.01) in T4 fed pigs than T1, T2, T3 and T5 and the total protein content was comparable among T2, T3 and T5 and the values were 5.41, 5.98, 5.96, 6.75 and 5.95, respectively for T1 to T5 pig feds. The Albumin (g/dl) content was not significantly different among the treatments and the values were 4.25, 4.41, 4.43, 4.15 and 4.26, respectively for T1 to T5 fed pigs. The Globulin (g/dl) and IgG (mg/dl) content were highest (P<0.01) in T4 fed diets and it was in order of T4>T5>T2>T3>T1 fed pigs and the values

were 1.16, 649.16; 1.57, 695.16; 1.52, 694.16; 2.60, 716.50 and 1.69, 697.00, respectively for T1 to T5 fed pigs.

The haematological parameters of crossbred pigs at the end of creep and grower phases were also studied and shown in Table 19 and 20. In pre-weaning animals there was no difference in PCV (%) among the treatments and the values were 33.74, 31.39, 31.56, 34.66, and 33.84 respectively for T1 to T5 fed pigs. The RBC count is higher ($P<0.01$) in pigs fed treatment diets when compared to control group and the values were 5.24, 7.76, 7.96, 8.06 and 7.98, respectivelyfor T1 to T5 fed pigs. WBC count was also significantly increased ($P<0.01$) in T4 fed pigs than in other treatments and the values were 18.00, 19.80, 19.56, 19.99 and 19.93 ($x10^3/\mu l$), respectively for T1 to T5 fed pigs.There was no significance difference in lymphocyte (%)and the values were 43.50, 43.50, 43.50, 46.50 and 45.50, respectively for T1 to T5 fed pigs.

The total protein (g/dl) content was significantly higher ($P<0.01$) in T4 fed pigs than T1, T2, T3 and T5 and the total protein content is comparable among T2, T3 and T5 and the values were 4.14, 4.95, 5.09, 5.31 and 5.09, respectively for T1 to T5 pig feds. There was no significant difference inalbumin (g/dl) content among the treatments and the values were 3.22, 3.52, 3.07, 2.95 and 2.99, respectively for T1 to T5 fed pigs. The globulin (g/dl) and IgG (mg/dl) content was higher ($P<0.01$) in T4 fed pigs and the values were 0.92, 630.50; 1.43, 678.50; 2.01, 685.00; 2.35, 700.00 and 2.10, 681.50, respectively for T1 to T5 fed pigs.

In growers also there was no difference in PCV (%) among the treatments and the values were 34.00, 34.14, 34.89, 35.67 and 33.05, respectively for T1 to

T5 fed pigs. The Hb (g/dl) content is higher (P<0.01) in pigs fed T4 diets than in other treatments and the values were 10.82, 12.01, 11.90, 12.99 and 11.81 respectively for T1 to T5 fed pigs. However, there was no significance difference in Neutrophil, Lymphocyte, Monocyte and Eosinophil count among different treatments.

The total protein (g/dl) content was significantly higher (P<0.01) in T4 fed pigs than T1, T2, T3 and T5 and the total protein content is comparable among T2, T3 and T5 and the values were 5.09, 5.23, 5.36, 5.92 and 5.21, respectively for T1 to T5 pig feds. The globulin (g/dl) and IgG (mg/dl) content was higher (P<0.01) in pigs fed experimental diets than control group and the values were 0.96, 645.50; 1.54, 690.00; 2.23, 698.00; 1.80, 708.50 and 1.29, 672.00, respectively for T1 to T5 fed pigs.

Table 19: Effect of treatments on haematological parameters in pre-weaned piglets

Parameter	T1	T2	T3	T4	T5
PCV (%)^{NS}	33.74±1.09	31.39±0.56	31.56±0.45	34.66±0.16	33.84±0.72
Hb (g/dl) **	10.58^{b}±0.03	10.05^{c}±0.06	10.10^{c}±0.05	11.12^{a}±0.02	11.00^{a}±0.11
RBC ($x10^{6}$/µl)**	5.24^{b}±0.73	7.76^{a}±0.04	7.96^{a}±0.02	8.06^{a}±0.05	7.98^{a}±0.01
WBC ($x10^{3}$/µl)**	18.00^{b}±0.31	19.8^{a}0±0.21	19.56^{a}±0.08	19.99^{a}±0.01	19.93^{a}±0.01
Neutrophils (%)*	49.00^{a}±2.0	41.00^{b}±1.00	49.00^{a}±2.00	50.50^{a}±0.50	51.00^{a}±1.00
Lymphocytes (%)^{NS}	43.50±0.5	43.50±1.50	43.50±0.50	46.50±0.50	45.50±0.50
Monocytes ^{NS}	4.50±1.5	4.00±1.00	4.50±1.50	2.00±1.00	2.50±0.50
Eosinophils ^{NS}	3.00±1.0	3.50±1.50	3.00±0.01	1.00±0.01	1.00±0.01
Basophils	0	0	0	0	0
Total protein (g/dl)**	4.14^{b}±0.02	4.95^{a}±0.06	5.09^{a}±0.03	5.31^{a}±0.18	5.09^{a}±0.02
Albumin (g/dl) ^{NS}	3.22±0.04	3.52±0.26	3.07±0.13	2.95±0.06	2.99±0.01
Globulin(g/dl)**	0.92^{c}±0.02	1.43^{b}±0.20	2.01^{a}±0.10	2.35^{a}±0.11	2.10^{a}±0.01
IgG (mg/dl)**	630.50^{b}±5.50	678.50^{a}±6.50	685.00^{a}±11.00	700.00^{a}±2.00	681.50^{a}±10.50

^{abcd}values in a row not sharing common superscripts differ significantly *(P<0.05) **(P<0.01) ^{NS} Not significant

Table 20: Effect of treatments on haematological parameters in growers

Parameter	T1	T2	T3	T4	T5
PCV (%)^{NS}	34.00±0.83	34.14±0.49	34.89±0.11	35.67±0.55	33.05±0.13
Hb (g/dl)**	10.82^{c}±0.27	12.01^{b}±0.10	11.90^{c}±0.21	12.99^{a}±0.01	11.81^{c}±0.17
RBC ($x10^{6}$/µl) ^{NS}	6.85±0.13	7.73±7.06	7.99±0.01	8.46±0.04	7.99±0.02
WBC ($x10^{3}$/µl)**	18.30^{a}±0.29	16.73^{c}±0.05	17.95^{b}±0.06	18.08^{a}±0.06	18.96^{a}±0.04
Neutrophils (%)^{NS}	50.50±0.50	49.50±0.50	50.00±2.00	49.50±1.50	48.50±0.50
Lymphocytes (%)^{NS}	41.50±2.50	44.00±1.00	43.00±1.00	44.50±1.50	46.50±0.50
Monocytes ^{NS}	4.00±1.00	3.50±0.50	4.50±0.50	3.50±0.50	3.50±0.50
Eosinophils ^{NS}	3.50±1.50	3.00±1.00	2.50±0.50	2.50±0.50	1.50±0.50
Basophils	0	0	0	0	0
Total protein (g/dl)**	5.09^{b}±0.07	5.23^{b}±0.04	5.36^{b}±0.05	5.92^{a}±0.03	5.21^{b}±0.09
Albumin (g/dl)**	4.12^{a}±0.05	3.69^{a}±0.07	3.13^{b}±0.20	4.12^{a}±0.06	3.92^{a}±0.06
Globulin(g/dl) **	0.96^{c}±0.01	1.54^{b}±0.02	2.23^{a}±0.25	1.80^{a}±0.10	1.29^{b}±0.03
IgG (mg/dl)**	645.50^{b}±1.50	690.00^{b}±3.00	698.00^{b}±1.00	708.50^{a}±1.50	672.00^{b}±2.00

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

Table 21: Effect of dietary treatments on haematological parameters in finishers

Parameter	T1	T2	T3	T4	T5
PCV (%)**	31.78^{b}±0.65	34.51^{a}±0.55	34.88^{a}±0.55	34.91^{a}±0.32	32.94^{b}±0.47
Hb (g/dl)**	10.73^{c}±0.20	11.94^{b}±0.42	12.27^{ab}±0.49	13.22^{a}±0.19	12.72^{ab}±0.14
RBC ($x10^{6}$/µl)**	6.10^{c}±0.44	7.60^{ab}±0.27	7.38^{b}±0.26	8.25^{a}±0.20	8.15^{ab}±0.21
WBC ($x10^{3}$/µl)^{NS}	15.54±0.66	16.25±0.54	16.25±0.54	16.62±0.69	16.26±0.48
Neutrophils (%)**	48.16^{b}±1.07	46.66^{c}±0.66	46.00^{c}±0.57	49.00^{ab}±0.36	50.33^{a}±0.49
Lymphocytes (%)**	43.16^{c}±0.94	45.50^{ab}±0.76	45.00^{bc}±0.68	47.16^{a}±0.47	45.66^{ab}±0.42
Monocytes**	4.83^{a}±0.60	5.00^{a}±0.63	5.66^{a}±0.49	2.83^{c}±0.54	3.16^{bc}±0.40
Eosinophils**	3.66^{a}±0.95	2.83^{a}±0.65	3.33^{a}±0.76	1.00^{b}±0.25	0.83^{b}±0.40
Basophils^{NS}	0	0	0	0	0
Total protein (g/dl)**	5.41^{c}±0.17	5.98^{b}±0.14	5.96^{b}±0.16	6.75^{a}±0.13	5.95^{bc}±0.28
Albumin (g/dl) ^{NS}	4.25±0.08	4.41±0.08	4.43±0.06	4.15±0.11	4.26±0.15
Globulin(g/dl)**	1.16^{c}±0.13	1.57^{bc}±0.15	1.52^{bc}±0.14	2.60^{a}±0.19	1.69^{b}±0.13
IgG (mg/dl)**	649.16^{d}±1.32	695.16^{c}±5.49	694.16^{c}±5.71	716.50^{a}±5.44	697.00^{b}±2.64

^{abc}values in a row not sharing common superscripts differ significantly **(P<0.01) ^{NS} Not significant

4.10. Effect of dietary treatments on serum triglycerides, HDL, LDL and total cholesterol

4.10.1. In piglets

The effects of dietary treatments on serum triglycerides, HDL, LDL and total cholesterol are shown in Table 22.In the present study dietary supplementation of shrimp shell meal containing chitosan not only decreased ($P<0.01$) the serum triglycerides and total cholesterol, but also reduced the LDL cholesterol and increased ($P<0.05$) the HDL cholesterol and the values were 76.25, 66.80, 41.00, 53.25; 70.75, 65.25, 43.00, 27.75; 69.00, 66.00, 42.25, 26.75; 64.25, 58.50, 45.00, 19.25; and 65.25, 61.75, 41.75, 23.50 of total cholesterol, triglycerides, HDL and LDL, respectively for T1 to T5 fed pigs.

4.10.2. In growers

The effects of dietary treatments on serum triglycerides, HDL, LDL and total cholesterol in growers are shown in Table 23. The total cholesterol and triglycerides levels were lower ($P<0.01$) in treatment groups when compared to control group and the values were 76.75, 72.25; 72.00, 68.00; 71.75, 68.25; 64.75, 60.25 and 69.00, 68.00 of total cholesterol and triglycerides, respectively for T1 to T5 fed pigs. The HDL cholesterol levels fed experimental diets were increased ($P<0.01$)and there was no significance difference in LDL cholesterol levels among the treatments and the values were 41.50, 35.25; 43.25, 28.75; 43.75, 28.00; 44.25, 20.50 and 44.25, 24.75 of HDL and LDL, respectively for T1 to T5 fed pigs.

4.10.3. In finishers

The effects of dietary treatments on serum triglycerides, HDL, LDL and total cholesterol in growers are shown in Table 24. The total cholesterol and triglyceride levels were significantly lowered ($P<0.01$) in pigs fed experimental diets than control group and the values were 77.33, 72.80; 75.33, 70.00; 74.08, 68.58; 67.40, 61.50; 71.08, 65.83 of total cholesterol and triglycerides, respectively for T1 to T5 fed pigs. The HDL levels were highest ($P<0.01$) in T4 fed pigs than T1, T2, T3 and T5 and the HDL levels of T2, T3 and T5 were comparable and significantly higher ($P<0.01$) than control group and the values were 43.08, 45.83, 46.16, 48.75 and 46.66, respectively for T1 to T5 fed pigs. The LDL levels were significantly lowest ($P<0.01$) in T4 fed pigs than T1, T2, T3 and T5 and LDL levels were highest in control group (T1) than other treatments.

Table 22: Effect of treatments on serum lipid profile (mg/dl) in pre-weanedpiglets

Parameter	T1	T2	T3	T4	T5
Total cholesterol**	$76.25^{a}\pm0.25$	$70.75^{b}\pm0.25$	$69.00^{b}\pm0.50$	$64.25^{c}\pm0.25$	$65.25^{c}\pm0.25$
Triglycerides**	$66.80^{a}\pm0.30$	$65.25^{a}\pm0.75$	$66.00^{a}\pm0.50$	$58.50^{c}\pm0.50$	$61.75^{b}\pm0.75$
HDL^{NS}	41.00±0.50	43.00±0.50	42.25±1.25	45.00±0.50	41.75±0.25
LDL*	$35.25^{a}\pm0.25$	$27.75^{b}\pm0.25$	$26.75^{b}\pm1.75$	$19.25^{c}\pm0.75$	$23.50^{bc}\pm0.50$

^{abc}values in a row not sharing common superscripts differ significantly **(P<0.01); *(P,0.05); ^{NS} Not significant

Table 23: Effect of treatments on serum lipid (mg/dl) profile in growers

Parameter	T1	T2	T3	T4	T5
Total cholesterol**	$76.75^{a}\pm0.25$	$72.00^{b}\pm0.50$	$71.75^{b}\pm0.25$	$64.75^{c}\pm0.25$	$69.00^{c}\pm0.50$
Triglycerides**	$72.25^{a}\pm0.75$	$68.00^{b}\pm0.50$	$68.25^{b}\pm0.25$	$60.25^{c}\pm0.75$	$68.00^{b}\pm0.50$
HDL^{NS}	41.50±0.50	43.25±0.25	43.75±0.25	44.25±0.75	44.25±0.75
LDL**	$35.25^{a}\pm0.25$	$28.75^{b}\pm0.25$	$28.00^{b}\pm0.50$	$20.50^{d}\pm1.00$	$24.75^{c}\pm0.25$

^{abc}values in a row not sharing common superscripts differ significantly **(P<0.01); *(P,0.05)

Table 24: Effect of treatments on serum lipid profile (mg/dl) in finishers

Parameter	T1	T2	T3	T4	T5
Total cholesterol**	$77.33^{a}\pm1.23$	$75.33^{ab}\pm0.30$	$74.08^{b}\pm0.45$	$67.40^{d}\pm0.46$	$71.08^{c}\pm0.71$
Triglycerides**	$72.80^{a}\pm0.71$	$70.00^{b}\pm0.65$	$68.58^{b}\pm0.43$	$61.50^{d}\pm0.34$	$65.83^{c}\pm0.96$
HDL**	$43.08^{c}\pm0.56$	$45.83^{b}\pm0.35$	$46.16^{b}\pm0.72$	$48.75^{a}\pm0.47$	$46.66^{b}\pm0.55$
LDL**	$34.25^{a}\pm0.72$	$29.50^{b}\pm0.46$	$27.91^{b}\pm1.04$	$18.65^{d}\pm0.34$	$24.41^{c}\pm0.23$

^{abc}values in a row not sharing common superscripts differ significantly **(P<0.01)

4.11. Effect of dietary treatments on muscle cholesterol and total fat

The effect of dietary treatments on muscle cholesterol and total fat are presented in Tables 25. The carcass fat was lower ($P<0.01$) in T4 fed pigs than in other treatments and the highest($P<0.01$) carcass fat was observed in control and the values were 12.35, 11.41, 10.60, 8.58 and 10.91 (g/100g), respectively for T1 to T5 fed pigs. The muscle cholesterol levels were also significantly lower ($P<0.01$) in T4 fed pigs, whereas cholesterol levels in T2, T3 and T5 fed pigs were lower ($P<0.01$) than in control group and the values were 94.91, 86.50, 82.66, 70.41 and 79.25 (mg/100g), respectively for T1 to T5 fed pigs. The same trend was also observed in growers too and the carcass fat and cholesterol levels were lower than those of pigs in the control and antibiotic fed groups and the values were 11.75, 88.75 ; 11.35, 86.75; 11,25, 85.25; 9.25, 78.25 and 10.75, 81.75 of carcass fat and cholesterol, respectively for T1 to T5 fed pigs.

Table 25: Effect of treatments on carcass fat and cholesterol content in finishers

Parameter	T1	T2	T3	T4	T5
Carcass fat (g/100g) **	12.35^{a} ±0.39	11.41^{ab}±0.23	10.60^{b}±0.45	8.58^{c}±0.22	10.91^{b}±0.35
Cholesterol (mg/100g) **	94.91^{a}±3.58	86.50^{b}±1.55	82.66^{bc}±0.47	70.41^{d}±1.86	79.25^{c}±1.79

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

4.12. Effect of dietary treatments on carcass characteristics

The carcass characteristics of pigs slaughtered at the end of grower phase were shown in Table 26. The weight at slaughter was in the range of 36.25 to 38.00 for all the treatments and it was significantly lower (P<0.01) in T4 than in other treatments. There was no significant difference in hot carcass weight and dressing percentage among treatments and the values were 29.27,79.12; 30.80, 81.05; 30.64, 82.25; 29.41, 81.13 and 29.99, 80.59, respectively for T1 to T5 fed pigs. Thecarcass length (cm) was significantly lower (P<0.01) in T1 and T2 and the values were 60.50, 63.00, 69.50, 70.50 and 69.50 for T1 to T5 fed pigs, respectively. The primal cuts which include ham weight (kg), loin weight (kg) and shoulder weight (kg) for T1 to T5 fed pigs were 9.06, 7.20, 5.83; 8.98, 7.86, 5.89; 9.26, 7.94, 6.45; 9.16, 7.58,6.21 and 9.18,7.65,6.47, respectively and there was no difference among treatments.The loin eye area (sq.cm) was significantly higher (P<0.05) in T5 than in other treatments and the values were 21.50, 21.25, 22.50, 21.50 and 23.50 for T1 to T5 fed pigs respectively. The average back fat thickness (cm) was significantly higher (P<0.05) in T1 than in other treatments and the values were 1.88, 1.70, 1.53, 1.46 and 1.55 for T1 toT5 fed pigs respectively.

The effect of dietary treatments on carcass characteristics of finisher pigs are presented in Table 27. The weight at slaughter was in the range of 70.66 to 71.58 kg for all the treatments and was not significantly different. The hot carcass weight was higher (P<0.05) in T4 (58.63) and T5 (58.18) fed pigs than in T1 (55.47), T2 (56.62) and T3 (57.94) fed pigs. The dressing percentage was significantly higher (P<0.01) in T4 (82.84) fed pigs when compared to T1 (78.40), T2 (80.11), T3 (81.54) and T5 (81.27) fed pigs. There was no significant

difference in carcass length (cm) among treatments and the values were 70.75, 70.66, 70.91, 70.66 and 71.08 for T1 to T5 fed pigs, respectively. But the loin area (sq cm) was significantly higher ($P<0.01$) in T4 (37.16) and T3 (37.0) fed pigs than T1 (29.50), T2 (32.0) and T5 (32.0) fed pigs while there was no significant difference in average back fat thickness (cm) among treatments and the values were 2.32, 2.24, 2.13, 2.09 and 2.24 for T1 to T5 fed pigs, respectively. The primal cuts which include ham weight (kg), loin weight (kg) and shoulder weight (kg) for T1 to T5 fed pigs were 14.79, 12.87, 9.53; 14.94, 13.95, 9.40; 15.08, 13.59, 9.57; 14.95, 13.67, 9.17 and 14.76, 13.74, 9.28, respectively and the differences among treatments were not significant.

Table 26: Effect of treatments on carcass characteristics of crossbred grower pigs

Treatment	Weight at slaughter (Kg)	Hot carcass weight (Kg)	Carcass length (cm)	Loin eye area (sq. cm)	Average back fat thickness (cm)	Primal cuts			Dressing percentage
						Weight of ham (Kg)	Weight of Loin (Kg)	Weight of shoulder (Kg)	
T1	37.00±0.50	29.27±0.26	60.50±1.50	21.50±0.50	1.88±0.08	9.06±0.74	7.20±0.38	5.83±0.75	79.12±0.35
T2	38.00±0.50	30.80±0.34	63.00±1.00	21.25±0.25	1.70±0.03	8.98±0.81	7.86±0.01	5.89±0.15	81.05±0.14
T3	37.25±0.25	30.64±0.84	69.50±0.50	22.50±0.50	1.53±0.08	9.26±0.14	7.94±0.17	6.45±0.32	82.25±1.71
T4	36.25±0.25	29.41±0.02	70.50±0.50	21.50±0.01	1.46±0.05	9.16±0.07	7.58±0.01	6.21±0.14	81.13±0.06
T5	37.25±0.25	29.99±0.17	69.50±0.50	23.50±0.50	1.55±0.05	9.18±0.15	7.65±0.06	6.47±0.35	80.59±0.08

[abc]values in a column not sharing common superscripts differ significantly *(P<0.05) **(P<0.01) [NS] Not significant

Table 27: Effect of dietary treatments on carcass characteristics of crossbred finisher pigs

Treatment	Weight at slaughter (kg)^{NS}	Hot carcass weight (kg)*	Dressing percentage**	Carcass length (cm)^{NS}	Loin eye area (sq. cm)**	Average back fat thickness (cm)^{NS}	Primal cuts		
							Weight of ham (kg)^{NS}	Weight of Loin (kg)^{NS}	Weight of shoulder (kg)^{NS}
T1	70.66±0.27	55.47^{c}±0.32	78.40^{c}±0.30	70.75±0.58	29.50^{b}±0.56	2.32±0.11	14.79±0.20	12.87±0.32	9.53±0.13
T2	70.66±0.42	56.62^{b}±0.50	80.11^{b}±0.50	70.66±0.71	32.00^{b}±1.12	2.24±0.09	14.94±0.10	13.95±0.81	9.40±0.13
T3	70.83±0.38	57.94^{ab}±0.69	81.54^{ab}±0.70	70.91±0.47	37.00^{a}±0.93	2.13±0.08	15.08±0.11	13.59±0.87	9.57±0.19
T4	70.75±0.38	58.63^{a}±0.71	82.84^{a}±0.63	70.66±0.49	37.16^{a}±0.60	2.09±0.10	14.95±0.23	13.67±0.53	9.17±0.12
T5	71.58±0.27	58.18^{a}±0.27	81.27^{ab}±0.55	71.08±0.41	32.00^{b}±1.12	2.24±0.03	14.76±0.27	13.74±0.15	9.28±0.14

^{abc}values in a column not sharing common superscripts differ significantly *(P<0.05) **(P<0.01) ^{NS} Not significant

4.13. Cost of feed per kg pork production

The cost of feed per kg saleable meat was calculated and it was higher ($P<0.01$) in pigs fed T1, T2 than those fed T3,T4 or T5 and the values (Rs.) were 108.94, 95.78, 84.14, 74.80 and 83.64 in pigs fed T1 to T5, respectively.

CHAPTER - V

DISCUSSION

Results of the present study are discussed with the observations of various authors and their publications relevant to utilization of shrimp shells (SSM) containing chitosan as a source of prebiotic in crossbred pigs.

5.1 Chemical composition of shrimp waste

The dry matter content of fresh shrimp shell waste was 29.0% and it contained 39.5% CP, 4.8% EE, 8.7% CF, 24.8% TA, 22.2% NFE, 7.18 % Ca, 3.45% P, and 15.5% chitosan. It was also rich in NDF, ADF and Silica (Table 2). The chemical composition of SSM as reported by various authors is presented in Table 1 and the chemical composition of the present study was comparable with the mean of the values reported by various authors. The CP, EE, CF, TA, Ca and P content of SSM in the present study on comparision with the mean of the values reported by various authors were 39.5 vs. 40.6; 4.8 vs. 5.2; 8.7 vs. 11.9; 24.8 vs. 20.9; 7.18 vs. 7.5 and 3.45 vs. 1.5, respectively.

5.2 Characterization of chitosan

The FT-IR spectra of extracted chitosan in the present study (Table 3 and Figure 1) showed the band spectrums of different bonds i.e. at 3851 cm^{-1} of OH group, 3432cm^{-1} for N-H stretching, 1628cm^{-1}, 1548cm^{-1} and 1373cm^{-1} for amide I, amide II and amide III bands, respectively. These observations were in close agreement with the reported values of 3858cm^{-1} for OH stretching, 3609cm^{-1} for N-H stretching, 1643 cm^{-1}, 1552 cm^{-1} and 1374 cm^{-1} for amide I, amide II and amide III bands, respectively in standard chitin (Figure 2) (Puvvada *et al.* 2012).

Furthermore, the observations of the current study were also consistent with the report of Kamala *et al.* (2013) who showed a peak at 3436 cm^{-1} indicating

presence of OH stretching and 1552 cm^{-1} indicating presence of amide 2 band, while 1322cm^{-1} and 778 cm^{-1} for amide III band and NH out of plane bending. Selimot *et al.* (2013) also reported 3439cm^{-1} for OH stretching, 1654cm^{-1} for stretching of amide bonds, stretching and vibrating of secondary amide at 1558cm^{1}, 1412cm^{-1} of CH_2 bending and CH_3 deformation and were also in line with the findings of present study.

The FTIR spectra of chitosan analysed by Arafat *et al.* (2015) showed a strong absorption band at 3454 cm^{-1} due to OH and NH symmetrical stretching vibrations similar to that recorded in the present study which showed NH stretching at 3432 cm^{-1} and also 1627 cm^{-1}, 1554 cm^{-1} and 1021 $cm^{-1}$of amide band 1, amide bond 2 and CO-stretchings showed were in agreement with the values of the present study (Table 3). Krishnaveni and Ragunathan (2015) reported bands at 3429 cm^{-1}, 1651 cm^{-1}, 1551 cm^{-1} and 1417 cm^{-1} for commercial chitosan that were in agreement with the present study. Ameh *et al. (*2015) observed absorbance bands at 3268, 1643, 1552, 1421, 1822, 893 and 752 cm^{-1}.

5.3. Ingredient and chemical composition of experimental diets

All the experimental diets were formulated as per NRC, 1998 standards.

5.3.1. Creep diets

The ingredient and chemical composition (%) of experimental creep diets used in the present study are presented in Table 4. During creep phase a total of 240 pre-weaned piglets were assigned at random to 5 dietary treatments of a basal control diet (T_1), T_1 supplemented with antibiotic (T_2), while in treatments T3, T4, and T5 dried and autoclaved SSM containing 15.5% chitosan was included at 0.5, 1.0 and 1.5% respectively. The resulting rations contained 700, 1500 and 2300 mg chitosan/kg.

All experimental diets were isonitrogenous. However, there was an increase in TA and CF and decrease in NFE in the diets T2 to T5 than in T1. Among the experimental diets, T5 contained maximum fiber (7.69) and total ash (10.01) that could be attributed to higher total ash in shrimp shell meal.

5.3.2. Grower diets

The ingredient and chemical composition (%) of the experimental grower diets are presented in Table 5. The negative control diet (T1) was formulated with maize, SBM and DORB while in positive control diet (T2) antibiotic was included while experimental diets T3 to T5 were formulated by including shrimp shell meal at 2.5, 5.0 and 7.5 %, respectively.

All the experimental grower diets were isonitrogenous. There was a gradual increase in TA, CF, EE, Ca and P with increasing level of SSM in the experimental diets which might be attributed to a higher total ash, crude fiber, fat, calcium and phosphorus content in shrimp shell meal.

5.3.3. Finisher diets

The ingredient and chemical composition (%) of the experimental finisher diets is presented in Table 6. The negative control diet (T1) was formulated with maize, SBM and DORB while in finisher positive control diet (T2) antibiotic was included. Experimental diets T3 to T5 were formulated by including shrimp shell meal at 2.5, 5.0 and 7.5 %, respectively.

All the experimental finisher diets were isonitrogenous. There was a gradual increase in TA, CF, EE, Ca and P with increasing levels of SSM in experimental diets which might be attributed to a higher total ash, crude fiber, fat, calcium and phosphorus content in shrimp shell meal.

5.4. Effect of experimental diets on small intestinal morphological structure

Small intestine is the main place for digestion and absorption of nutrients, and the intestinal mucosa plays an important role in these processes. Weaning stress can result in relatively quick changes in the intestinal mucosa morphology that may lead to villus atrophy and crypt hyperplasia (Xiong *et al.* 2015; Wan *et al.* 2017). The abnormal intestinal morphology is usually associated with retarded growth of weanling piglets. Shortening of the villus decreases the surface area for nutrient absorption, which lead to poor nutrient absorption and reduced performance (Xu *et al.* 2003). The crypt is the area where stem cells divide to permit the renewal of the villus, and a large crypt depth indicates fast tissue turnover and a high demand for new tissue (Hu *et al.* 2012). The ratio of villus height to crypt depth is a useful criterion for estimating the digestive capacity in the small intestine (Masri *et al.* 2015).

5.4.1. In pre-weaned piglets

The effect of dietary treatments on small intestinal morphology of piglets is presented in Table 7 and Figs. 3 to 8. There was a significant increase ($P<0.01$) in the height of villi in duodenum of piglets fed T4 ration compared to T2, T3 and T5 fed pigs also the same trend was noticed when compared to control group. The crypt depth was also decreased ($P<0.01$) in T4 fed pigs when compared to other treatments. And the ratio of villus height to crypt depth in duodenum was highest in T4 fed pigs, which was the major criteria to evaluate small intestinal maturity and health in pigs and ratios were in order of T4>T2>T5>T3>T1 fed pigs and the differences were significant ($P<0.01$).

Similarly, the height of villi in jejunum of pigs fed T4 ration was the highest ($P<0.01$) when compared to other treatments. The height of villi in

jejunum also increased in all other treatments when compared to negative control group and the differences were significant (P<0.01). The crypt depth in jejunum of pigs fed T4 ration was significantly lower (P<0.01) than other treatments. The ratio of villus height and crypt depth was also highest in T4 fed pigs and was in order of T4>T2>T5>T3>T1 fed pigs and the difference was significant (P<0.01).

The height of villi in ileum of pigs fed T4 ration was also significantly higher (P<0.01) in T4 and T5 fed pigs than other treatments. The crypt depth was the lowest (P<0.01) in T4 fed pigs than other treatments. The ratio of villus height to crypt depth was also highest (P<0.01) in pigs fed T4 ration. The ratio of villus height to crypt depth was in order of T4>T2>T5>T3>T1 fed pigs and the differences were significant.

The observations of present study reinforce the claims of previous authors Liu *et al.* (2008), Khambulai *et al.* (2009), Yang *et al.* (2012), Xiao *et al.* (2013), Xu *et al.* (2013), Swiatkiewicz *et al.* (2015) and Suthongsa *et al.* (2017) that feeding of chitosan has positive effect in improving the morphology.

Liu *et al.* (2008) performed a study aimed at evaluating the effects of diet supplementation with COS and reported that enhanced growth performance in weaned pigs was due to improvement of small intestine morphology. A total of 50 weaning pigs were selected and assigned randomly to 5 treatments including control, 3 diets with COS supplementation (100, 200 and 400 mg/kg) and another diet with chlortetracycline (CTC) (80 mg/kg) and reported supplementation of COS at 100 and 200 mg/kg and supplementation of CTC improved (P<0.05) growth performance by increasing the villus height and villus : crypt ratio in the jejunum of weanling pigs and concluded that COS could be an effective alternative to the use of antibiotic growth promoters.

Similar trend was observed in the present study also where in supplementation of shrimp shell waste containing chitosan had significant ($P<0.01$) effect comparable with antibiotics supplemented piglets in improving small intestine morphology.

Xiao *et al.* (2013) observed increase ($P<0.05$) in villus length and villus height, but villus width and crypt depth were decreased ($P<0.05$) both in COS and chlortetracycline fed groups. They observed that chitosan and the antibiotics have similar effects in promoting piglet growth by improving morphology of small intestine and concluded that chitosan could replace chlortetracycline as a feed additive for piglets.

Xu *et al.* (2013) reported that diets supplemented with increasing levels of chitosan increased quadratically the height of villi and the ratio of villus height to crypt depth in duodenum, jejunum and ileum on day 14 and day 28 and concluded that chitosan could quadratically improve growth in weaned pigs due to improvement of the small intestines morphology and increase of serum growth hormone concentration.

Swiatkiewicz *et al.* (2015) reported that chitosan increased quadratically the ratio of villus height to crypt depth in duodenum, jejunum and ileum on day 14 and decreased crypt depth in duodenum, jejunum and ileum on day 28. They concluded that chitosan could quadratically improve growth in weaned pigs, and the underlying mechanism was an increase of the serum GH concentration and improvement of the small intestines morphology.

Suthongsa *et al.* (2017) demonstrated that COS inclusion can improve growth performance through enhancing health by a combination of increased

nutrient bioavailability and increased gut absorption and function capacity associated with the elongated villi in duodenum, jejunum and ileum.

On the other hand, results of the present study disagreed with that of Yang *et al.* 2012 who observed no changes in villus height and crypt depth in duodenum, jejunum and ileum of small intestine when COS was included in the diets of weanling pigs and reported that improved growth performance was due to increased feed intake, increased nutrient digestibility and increased serum GH and IGF-1 concentrations.

One possible explanation related to the increased villus heights with dietary COS supplementation could be that of *N*-acetylglucosamine, which is a common component of receptor-active oligosaccharides (Suthonsga *et al.* 2017). *N*-acetylglucosamine is a basic component of COS (Kim and Rajapakse, 2005). Previous studies revealed that the *N*-acetylglucosamine is a common component of many mammalian glycoconjugates, particularly of mucins (Klemm and Schembri, 2000; Ofek *et al.* 2003; Podolsky, 1985), which could be used as receptors for preventing a wide range of bacteria from binding to the gut tissue of host animals. Thus, the *N*-acetylglucosamine abundance in COS may cause binding of COS to certain types of bacteria (Klemm and Schembri, 2000; Ofek *et al.* 2003), possibly interfering with their colonization in the gut (Stanley *et al.* 2000; Rhoades *et al.* 2006). A decrease in intraluminal bacterial population has been shown to improve proliferation of epithelial cells to increase villi height in the gut (Mourão *et al.* 2006), thereby leading to enhanced intestinal morphology. Thus, the increased villus heights with dietary COS supplementation may lead to enhanced nutrient digestibility in weaned pigs (Pluske *et al.* 1996).

5.4.2 In growers

The effect of dietary treatments on small intestinal morphological feachers in growers is presented in Table 8 and in Figs 9 to 11. Weaning stress leads to villus height decrease and crypt depth increase, in association with a reduction in the nutrient digestion and absorption capacity (Montagne *et al.,* 2003). Consequently, maintaining intestinal structure and morphological properties for enhanced digestion and absorption of nutrients after weaning is important. In the present study, greater villus height in the duodenum, jejunum and ileum was observed in pigs fed SSM compared to control group. This enhanced small intestinal morphology in early stages of growth period might have helped in achieving higher growth rate during grower and finisher phases also due to SSM inclusion at 5 to 7.5 % in the diets. The COS present in SSM could have modulated intestinal morphology and therefore enhanced nutrient absorption even at the higher levels of inclusion in later stages, which was in agreement with established literature (Yang *et al.* 2012; Xu *et al.* 2013; Wan *et al.* 2016; Wan *et al.* 2017).

There was a significant increase ($P<0.01$) in height of villi and decrease in crypt depth in duodenum of piglets fed T4 ration in the present study when compared to other treatments and the ratio of villus height to crypt depth in duodenum was highest in T4 fed pigs when compared to other treatments which was the major criteria to evaluate small intestinal maturity and health in pigs and ratio of villus height to crypt depth was in order of T4>T2>T5>T3>T1 fed pigs and the differences were significant ($P<0.01$).

Similarly, the height of villi in jejunum of pigs fed T4 was the highest ($P<0.01$) when compared to other treatments and the increase in height was in the

order of T4>T5>T2>T3>T1 fed pigs and the differences were significant (P<0.01). The crypt depth in jejunum of pigs fed T4 ration was significantly lower (P<0.01) than in other treatments. The ratio of villus height and crypt depth was also wider in T4 fed pigs and was in order of T4>T2>T3>T5>T1 fed pigs and the differences were significant (P<0.01).

The height of villi in ileum was higher and the crypt depth was lower (P<0.01) in T4 fed pigs in the present study leading to the wider ratio of villus height to crypt depth than in other treatments. The ratio of villus height to crypt depth was in order of T4>T2>T3>T5>T1 fed pigs and the differences were significant (P<0.01). The changes noticed in the small intestinal morphology in grower phase were similar to that of pre weaned animals.

5.4.3. In finishers

The effect of dietary treatments on small intestinal morphology in finishers are presented in Table 9 and from Figs 12 to Fig. 14 and similar trend was noticed even in finisher phase.

There was a significant increase (P<0.01) in height of villi in duodenum of pigs fed T4, T2, and T5 than those fed T3 and T1 and also decreased (P<0.01) crypt depth in T4 fed pigs was observed when compared to other treatments. And the ratio of villus height to crypt depth in duodenum was wider in T4 fed pigs when compared to other treatments and was in order of T4>T2>T5>T3>T1 fed pigs and the differences were significant (P<0.01).

Similarly, the height of villi in jejunum of pigs fed T4 and T5 ration were higher (P<0.01) when compared to other treatments and the differences were significant (P<0.01). The crypt depth in jejunum of pigs fed T4 ration was significantly lower (P<0.01) than in other treatments. The ratio of villus height to

crypt depth was also wider in T4 fed pigs and was in order of T4>T2>T5>T3>T1 fed pigs and the differences were significant (P<0.01).

The height of villi in ileum of pigs fed T4 ration was also highest (P<0.01) than in other treatments. The crypt depth was the lowest (P<0.01) in T4 fed pigs than other treatments. The ratio of villus height to crypt depth was wider (P<0.01) in pigs fed T4 ration. The ratio of villus height to crypt depth was in order of T4>T2>T5>T3>T1 fed pigs and the differences were significant (P<0.01). The changes noticed in the small intestinal morphology in finisher phase were also similar to that observed during pre weaned or grower stages.

5.5. Effect of dietary treatments on pathogenic bacteria of large intestinal contents

Many studies have reported that different oligosaccharide preparations have antibacterial effect. For example, Smiricky-Tjardes *et al.* (2003) suggested that galactooligosaccharides can increase the concentration of beneficial bacteria, such as *Lactobacilli,* in the intestines of growing pigs. Han *et al.* (2007); Liu *et al.* (2008); Wang *et al.* (2009) and Yang *et al.* (2012) reported that fecal *Lactobacillus* population was increased while *E. coli* was decreased in the guts of weanling pigs when COS was used at different levels in the feed. Similarly, Li *et al.,* (2007); Kong *et al., (*2014) reported that COS supplementation could decrease fecal *E. coli* without impacting the concentration of *Lactobacilli* in the guts of growing broiler chickens and weaned Huanjiang mini piglets, respectively.

The results of the present study (Tables 10, 11 and 12) were in agreement with the above mentioned studies. In the present study also there has been a significant reduction (P<0.01) in the *E.coli* and *Salmonella* count (cfu/g) in the

large intestines of pigs fed shrimp shell meal containing chitosan than control group. Though the exact reason for the antimicrobial activity of COS remains unknown, Wang *et al. (*2009) suggested that the COS might affect the colonization of *Salmonella* strains by blocking bacterial attachment to the gut mucosa, thereby reducing the prevalence of *Salmonella*. Benhabiles *et al.* (2012) suggested two main mechanisms as the cause of the inhibition of microbial cells by chitosan. One was that the polycationic nature of chitosan interferes with bacterial metabolism by electrostatic stacking at the cell surface of bacteria. The other was blocking of transcryption of RNA from DNA by adsorption of penetrated chitosan to DNA molecules.

Yang *et al. (*2012) explined that a positive charge on the NH^{3+} group of the glucosamine monomer of COS allows interactions with negatively charged microbial cell membranes that lead to leakage of intracellular constituents (Liu *et al.* 2004). Another possible explanation is an indirect effect by increasing the populations of *Bifidobacteria* and *Lactobacilli*, and their subsequent competitive exclusion of *S. aureus.*

5.6. Metabolism trial during finisher phase

5.6.1. Digestibility of nutrients

The nutrient digestibility (%) of experimental diets during finisher phase is presented in Table 13. In the current study, digestibility of major nutrients except EE increased when pigs were offered diets containing shrimp shell meal. The digestibility of DM in pigs fed T4 and T5 was significantly higher ($P<0.05$) than those fed T1, T2 and T3 rations, whereas in T1, T2 and T3 fed pigs it was not significantly different among treatments. The CP digestibility was significantly

higher (P<0.01) in T4 fed pigs than in other treatments and also there was no significant difference in the digestibility of CP among other treatments. The CF and NFE digestibilities were higher (P<0.01) in pigs fed T4 and T5 rations than those fed T1, T2 and T3 fed rations, while the digestibility of EE was significantly lower (P<0.01) in pigs fed T4 and T5 rations than those fed T1, T2 and T3 fed rations.

The observations in the present study were in agreement with the previous studies of Hou and Gao. (2001); Tuohy *et al.* (2003); Huang *et al.* (2005); Lim *et al.* (2006); Han *et al.* (2007); Li *et al.* (2007); Liu *et al.* (2008); Chen *et al.* (2009); Wang *et al.* (2009); Yan and Kim (2011); Xu *et al.* (2014); Swiatkiewicz *et al.* (2015); Gandra *et al.* 2016 and Wan *et al.* (2017), which indicated that dietary supplementation of chitosan was effective in increasing digestibility of nutrients in pigs and this could be due to the higher digestive enzyme activities and enhanced intestinal morphology (Wan *et al.* 2017). Nonetheless, a little effect on nutrient digestibility and a tendency to improve ADG and ADFI in weaned pigs was observed by Chen *et al.* (2009).

Though the mechanism of action of chitosan is still not clear, the most acceptable theory (Kong *et al.* 2010) has been the interactions between positively charged chitosan amino group and negatively charged bacterial surface resulting in alteration of membrane permeability. Zhong *et al.* (2008) reported that gram-positive bacteria are more susceptible to derivaties of chitosan as a consequence of the gram-negative outer membrane barrier. The increase in protein digestibility could be related to the action of chitosan on bacteria which promote proteolysis and deamination, resulting in a decrease of ruminal protein degradation and increasing amino acids availability in the intestine, a similar effect of ionophores

(Yang and Russell, 1993). Mingoti *et al.* (2016) suggested that the increase of the nutrient digestibility with chitosan addition might be due to its capacity to change rumen microorganisms and digestive processes, acting mainly on the gram-positive bacteria, that is justified by improvement in CP digestibility. Previous studies with increasing doses of chitosan reported similar results of improved nutrient digestibility (Araujo *et al.* 2015; Paiva *et al.* 2016) and the effects were probably related to altered ruminal fermentation.

The present experiments also showed that in diets containing SSM there was a significant decrease in the apparent digestibility of fat. This observation is supported by previous study of Walsh *et al.* (2013) who reported the mechanism may be postulated as follows: Chitosan might interrupt enterohepatic bile acid circulation. Bile salt is mixed with dietary lipids and emulsifies the lipid particle, and the lipid particle size is reduced. The smaller particle size allows for greater surface exposure to pancreatic and intestinal lipase which is adsorbed on to the particle surface, and lipase activity is stimulated (Kobayashi *et al.* 2002). Chitosan which differs from other dietary fibres because of its cationic characteristics can reduce fat absorption and interrupt enterohepatic bile acid circulation by electrostatic and hydrophobic forces (Sumiyoshi and Kimura 2006; Aranaz *et al.* 2009). Secondly, chitosan might increase the viscosity of the intestinal contents. It has been generally accepted that the anti-fatty effects of chitosan originate from its unique fat binding properties. Dietary chitosan dissolves in the stomach, emulsifying fat and forming a gel, which binds with the fat in the intestine (Gades and Stern, 2003; Zeng *et al.* 2008; Zhang *et al.* 2008), increasing the viscosity of the intestinal contents and the unstirred layer in the intestine and slowing nutrient diffusion, which resulted in a highly effective increase in the excretion of fat

(Santas *et al.* 2012). Therefore, the inhibitory effect of chitosan on lipase activity may be attributed to the viscosity of chitosan which might have restricted the access of the pancreatic lipase to the lipids within the droplets and the reduction of bile acid concentration in the intestinal tract. Thirdly, chitosan has profound impact on circulating adipocytokines (*e.g.* leptin, resistin, IL-6, and C-reactive protein, etc.), which significantly suppress appetite, regulate energy metabolism, and prevent lipid accumulation in peripheral tissues (Unger., 2003a,b) and therefore chitosan can lower fat mass, regulate the level of circulating triglycerides, and reduce the accumulation of lipids both in the liver and in the muscle tissue (Neyrinck *et al.* 2009; Liu *et al.* 2012; Walsh *et al.* 2013). But, massive ingestion of chitosan might lead to a deficiency of fat-soluble vitamins such as vitamin E (Deuchi *et al.* 1995) as well.

5.6.2. N balance

The nitrogen balance of pigs fed experimental diets during finisher phase in the present study is presented in Table 14. The nitrogen intake (g/d) was higher ($P<0.01$) in pigs fed T1 than in other treatments and was lowest in pigs fed T4 ration and it was due to differences in feed intake during metabolism trial. Total nitrogen (g/d) loss was highest ($P<0.01$) in T1 fed pigs than in T2, T3, T4 and T5 fed pigs and it could be attributed to the higher CP digestibility in pigs fed experimental diets than control diet. The nitrogen retention (g/d) was lowest ($P<0.05$) in pigs fed T1 than pigs fed other experimental diets, which is a function of the digestibility, quality of the protein, amount of fed in relation to the requirements and other diet constituents (Whittemore, 1992). The nitrogen retention as % absorbed was comparable among the treatments and there was no significant difference among the treatments.

5.7. Growth performance

There have been several attempts to study the use of different types of oligosaccharides as a potential alternative antibiotic in enhancing animal growth (Halas and Nochta, 2012). Collins and Gibson (1999) provided an overall review of the benefits of oligosaccharides, including *N*-acetylglucosamine, oligofructose, lactulose and certain glycoproteins as prebiotics to improve gut health. Furthermore, several supplemental oligosaccharides, such as galacto-oligasaccharide and mananoligosaccharide, have been shown to improve growth performance in young pigs (Davis *et al.* 2004; Miguel *et al.* 2006; Halas and Nochta, 2012). However, there is still little knowledge of the possible benefits of COS. In fact, COS is the second most abundant carbohydrate polymer in nature (Knaul *et al.* 1999).

In the present study, dietary supplementation of shrimp shell meal containing chitosan, as well as supplementation of antibiotic (CTC) at 80 mg/kg have shown positive effects in improving the growth performance of pigs in different stages.

5.7.1. Creep phase (upto 42 days)

Growth performance of crossbred pigs during creep phase is presented in Table 15. The initial body weight, final body weight, number of days and average daily feed intake were not significantly different among treatments. The weight gain (kg) was higher ($P<0.01$) in piglets fed T3 toT5 than those fed T1 or T2. A superior ($P<0.01$) feed conversion ratio was observed in T4 than in T1 to T3 fed pigs and was comparable with T5 fed pigs. The feed intake was not affected and was comparable among treatments. The results of the present study are in agreement with published literature (Liu *et al.* 2008; Yang *et al.* 2012; Yuan and

Chen, 2012; Xu *et al.* 2013; Xu *et al.* 2014; Swiatkiewicz *et al.* 2015) that chitosan in pig diets promoted better growth performance. The cost of feed/kg gain was significantly lower ($P<0.01$) in T2 to T5 fed pigs than in control ration fed pigs. Overall, the least cost of feed/kg gain was observed in T4 fed pigs.

Several factors might have involved in improved performance of piglets. The small intestine is the main place for digestion and absorption of nutrients, and the intestinal mucosa plays an important role in these processes. Weanling stress can result in relatively quick changes in the intestinal mucosa morphology, which lead to a reduction in villus height and an increase in crypt depth (Hampson, 1986; Pluske *et al.* 1996). A shortening of the villus decreases the surface area for nutrient absorption, which lead to poor nutrient absorption and reduced performance (Xu *et al.* 2003). The ratio of villus height to crypt depth is a useful criterion for estimating the digestive capacity in the small intestine (Montagne *et al.* 2003). In agreement with the improved performance in weanling piglets, feeding of chitosan resulted in an improvement of intestinal morphology, as indicated by the increased small intestinal villus height and the ratio of villus height to crypt depth, and the decreased small intestinal crypt depth of weaned pigs in this present study. Similar results were also reported by other investigators (Liu *et al.* 2008; Yang *et al.* 2012; Xiao *et al.* 2013; Xu *et al.* 2013; Xu *et al.* 2014; Swiatkiewicz *et al.* 2015).

It is also well known that pathogenic germs such as coliforms can destroy the normal morphology of small intestinal mucosa. Furthermore, chitosan provided a beneficial environment for the proliferation of enterocytes, preventing intestinal atrophy (Han *et al.* 2007; Liu *et al.* 2008; Wang *et al.* 2009; Benhabiles *et al.* 2012; Yang *et al.* 2012; Kong *et al.* 2014). These studies showed that

chitosan was an effective polysaccharide in ameliorating intestinal structure and function, which may be one of the reasons for the increased growth performance in weaned pigs treated with chitosan.

It is well known that one of the major reasons for the improvement of growth performance was the increase in apparent digestibility of major nutrients. In the current study, apparent digestibility of major nutrients except fat was increased when pigs were offered increasing levels of shrimp shell meal containing chitosan. This result was in agreement with that of previous studies which indicated that dietary supplementation of chitosan was effective in increasing apparent total tract digestibility of nutrients (Lim *et al.* 2006; Han *et al.* 2007; Liu *et al.* 2008; Chen *et al.* 2009; Wang *et al.* 2009; Zhou *et al.* 2012; Xu *et al.* 2014).

Furthermore, although we did not study the plasma GH and IGF-I concentrations in the present study, Tang *et al.* (2005) found that early weaned pigs fed a diet containing COS at a concentration of 0.25 g/kg had increased plasma GH and IGF-I concentrations, which have led to improved growth. And it has also been suggested that the improved growth performance observed in response to dietary COS supplementation occurs via an increased feed intake (Tang *et al.* 2005). However, in the current study, feed intake was not increased and it could be assumed that such a discrepancy might be due to different species/breeds (Xu *et al.* 2014).

5.7.2. Grower phase (15 to 35 kg body weight)

The growth performance of pigs during grower phase (15 to 35 kg) is presented in Table 16. The initial weight, final weight and weight gain (kg) were comparable among treatments. The number of days taken to attain the final body

weight was lower (55 days) ($P<0.01$) in T4 fed pigs when compared to pigs fed control diets (72 days) and also the number of days taken to attain final weight was higher ($P<0.01$) in pigs fed control diet when compared to T2, T3 and T5. The average daily gain was highest ($P<0.01$) in T4 fed pigs and was in the order of T4>T5>T3>T2 and T1 fed pigs. The ADFI (kg) was significantly higher ($P<0.01$) in T4 and T5 fed pigs and ADFI was comparable among T1, T2 and T3. The feed per kg gain and cost of feed per kg gain (Rs.) were highest ($P<0.01$) in T1 fed pigs when compared with those of T2, T3, T4 and T5.

The results of present study were in agreement with earlier studies of Yan and Kim (2011) and Xu *et al.* (2013) who showed beneficial effects of supplementing chito-oligosaccharides in promoting higher ($P<0.01$) growth rate and feed efficiency in growing pigs. Similar to the trend observed in creep phase the increased growth rate in grower phase may be attributed to improved intestinal morphology, reduction in gut pathogenic bacteria and increased digestibility of nutrients, apart from increasing feed intake, which were in line with the findings of Yuan and Chen (2012). However, Walsh *et al.* (2013) observed that dietary supplementation of shrimp shell / chitosan reduced body weights and feed intake when compared with those fed on control diets. As a possible explanation for these divergences the authors hypothesized that in different experiments different doses of chitosan had been used.

5.7.3. Finisher phase (35 to 70 kg body weight)

During finisher phase (Table 17) the initial and final body weights and weight gain (kg) were not significantly different among treatments. Similar to the trend observed in grower phase, the pigs fed T4 had taken less number of days ($P<0.01$) than in other treatments and pigs fed control diet had taken maximum

number of days to reach the target weight. The ADG (g) was significantly higher (P<0.01) in T4 followed by T5 than in other treatments. The ADFI (kg) was not significantly different among treatments. The feed/kg gain was lower (P<0.01) in pigs fed T4 diets followed by T5, T3 and T2 than in T1leading to a lower cost of feed per kg gain (Rs.) in pigs fed T4 diets and the cost of feed per kg gain was in order of T4<T5<T3<T2<T1 fed pigs.

5.7.4. Overall growth performance (15 to 70 kg body weight)

The overall growth performance of pigs covering grower and finisher phases is presented in Table 18. The initial body weight, final body weight and weight gain (kg) were not significantly different among treatments. The pigs fed on T4 have taken less (P<0.01) number of days to attain the target weight than in T5, T3, T2 and T1. The pigs fed control diet *i.e.* T1 have taken maximum (P<0.01) number of days to attain the target weight. The ADG (g) was higher (P<0.01) in T4 followed by T5 than in other treatments. The ADFI (kg) was not significantly different among the treatments. The feed per kg gain was lower (P<0.01) in T4, T5 or T3 fed pigs than in T2 or T1. The cost of feed per kg gain (Rs.) was significantly lower (P<0.01) in T4, T5, T3 than in T2 or T1 fed pigs.

The better performance of pigs fed T3 to T5 diets could be attributed to improved intestinal morphology, reduction in gut pathogenic bacteria and increased digestibility of nutrients which are in agreement with earlier observations of Huang *et al.* (2005); Shi *et al.* (2005); Han *et al.* (2007); Kim *et al.* (2008); Liu *et al.* (2008); Khambualai *et al.* (2009); Yang *et al.* (2012); Yuan and Chen (2012); Xu *et al.* (2013); Xu *et al.* (2014), Swiatkiewicz *et al.* (2015) and Suthongsa *et al.* (2017).

5.8. Effect of dietary treatments on haematological parameters

The blood profiles of animals reflect the physiological disposition of their nutrition according to their internal and external environments. Therefore in the present study we measured these characteristics to determine the response by which SSM influenced the pigs. In the present study (Table 19, 20 and 21), serum total protein concentration was increased ($P<0.01$) in response to SSM in diets, which indicated that the protein status of the pigs had improved. The increased total protein concentration was primarily due to an improved globulin concentration, since there was increase in the serum IgG concentration whereas the albumin concentration was not significantly different among dietary treatments. This response indicated that SSM had beneficial effects on the immune system.

Previous studies (Xiao *et al.* 2013; Wan *et al.* 2017) have demonstrated that dietary COS supplementation enhanced cell-mediated immune response in early weaned pigs by modulating the production of antibodies and by increasing the serum IgG levels, indicating that COS supplementation efficiently elicited a humoral immune response, which may provide an advantage in enhancing weaned pig immunity. In addition in diets T3 to T5 the lymphocyte concentration was also increased ($P<0.01$), which may indicate that COS had beneficial effects on the immune system which was in agreement with Okamoto *et al.* (2003); Wang *et al.* (2009); Eze *et al.* (2010) and Yan and Kim (2011).

In the current study, there was also a significant increase ($P<0.01$) in PCV (%), Hb (g/dl) and RBC ($x10^6$/µl) count in pigs fed rations containing shrimp shell meal when compared to control group. Togun *et al.* (2007) observed that increase

in PCV coupled with marginal increase in RBC is indicative of more efficient erythropoiesis in the experimental animals.

5.9. Effect of dietary treatments on serum triglycerides, HDL, LDL and total cholesterol

Among the natural products, many types of non-digestible dietary fibers were found to possess hypolipidemic activity. There is a considerable interest in the hypolipidemic activities of dietary non-nutritive substance. Chitosan is one of the known substances to affect the levels of lipids in serum. The beneficial effect of chitin-chitosan as a feed supplement in reducing plasma cholesterol and triglycerides was attributed to its ability to bind dietary lipids, thereby reducing intestinal lipid absorption. The hypolipidimic influence of chitosan may also be due to interruption of the enterohepatic bile acid circulation. Chitosan acts by forming gels in the intestinal tract which entrap lipids and other nutrients, including fat soluble minerals and vitamins, thus interfering with their absorption (Koide, 1998).

Our results (Table 22, 23 and 24) reported in the present study are in good agreement with results obtained in previous studies of Razdan and Pattersos (1994, 1996); Durdi Qujeq and Gholamreza Ataei (2000); Ma *et al.* (2001); Tang *et al.* (2005); Rauw *et al.* (2009); Zhang *et al. (*2012) and Gronde *et al.* (2016) where in addition to decreased ($P<0.01$) total cholesterol, triglycerides and LDL cholesterol, COS increased HDL cholesterol levels in serum. In the present study, there was a decrease ($P<0.01$) in serum total cholesterol, serum triglycerides, LDL cholesterol and increased HDL cholesterol levels suggesting that COS alter the serum lipid profile by either decreasing ($P<0.01$) the bad cholesterol (LDL), total cholesterol or serum triglycerides or by increasing the good cholesterol.

There are many studies attempting to explain the mechanisms of chitosan in inhibiting hyperlipidemia. Durdi Qujeq and Gholamreza Ataei (2000) reported that chitosan may affect cholesterol absorption both by binding cholesterol and presumably by disrupting micelle formation in the intestine. Consequently, chitosan might cause improvement of the fatty liver and hyperlipidaemia through inhibiting intestinal absorption of dietary fat. Therefore, it is likely that cholesterol was not absorbed by the proximal region of the small intestine, the site of cholesterol absorption, accumulated in the cecum which may explain decreased serum and liver cholesterol levels. Serum HDL-C level was increased significantly by the SSM feeding in comparison with control feeding. Also, serum LDL-C level was decreased by the chitosan feeding, which was attributed to the general reductions in serum cholesterol concentrations, probably caused by enhanced reverse cholesterol transport in response to intestinal losses of dietary fats. These results are also confirm findings of Razdan and Pattersos (1996).

Ma *et al.*, 2001 reported that COS can form a gel complex with gastric acid in the gastrointestinal tract where the gel complex cannot be degraded under the high pH environment in the intestine. This gel can absorb bile acid and cholesterol and the gel, bile acid, cholesterol mixtures are discharged in feces, thus the absorption of fat and cholesterol is decreased. The same mechanism for the decreased levels of triglycerides and cholesterol was also reported by Zhen *et al.* (1995) in their previous studies. Tang *et al.* 2005 and Zhang *et al.* 2012 suggested the reason for this response was that COS formed a gel complex with gastric acid in the gastrointestinal tract and was subsequently excreted in the feces.

Gronde *et al.* 2016 reported that chitosan acted in several ways. First, where chitosan is soluble, it lowers cholesterol levels by increasing the viscosity of stomach contents, which inhibits uptake of cholesterol. Next, chitosan acts as a cationic polysaccharide in an acidic environment, e.g. the stomach, so the positive amino groups of the fibre binds to negatively charged molecules, such as bile acids and fatty acids (Anandan *et al.* 2013). This leads to higher activity of the LDL-receptor and thus lowers LDL-C plasma levels (Santas *et al.* 2012). In the intestine, a higher P^H makes the complex precipitate with bound fatty and bile acids and cholesterol (Zhang *et al.* 2008). After precipitation, the bound fatty and bile acids are inaccessible to enzymes and are excreted with faeces. Another way of action could be the inhibition of pancreatic lipase activity (Choi *et al.* 2012).

In the present study, the SSM containing chitosan was clearly found to be considered the most powerful hypolipidemic natural product, because of these considerations together with its low toxicity, chitosan seems to be a realistic hypocholesterolemic agent. Possibly, the general hypolipidemic action of chitosan will make this polycationic polymer the new important target of nutritional and biochemical studies.

5.10. Effect of dietary treatments on muscle cholesterol and total fat

Enough research is not done so far in this regard, however in the present study inadverdantly positive results (Table 25) were observed with supplementation of shrimp shell meal on muscle cholesterol and total fat. These observations are in comparision with that of Kim *et al.* (2008) where in it was reported that addition of COS in the diets of finishing pigs reduced the cholesterol in pork without affecting its quality. Zhou *et al.* (2009) observed that supplementation of COS in broiler chickens improved the quality of meat. In the

present study, T3 to T5 fed pigs, there was decrease (P<0.01) in muscle cholesterol and total fat in finisher pigs and the same trend was also observed in pigs fed grower rations suggesting that SSM owing to its chitosan content has positive effect in lowering the muscle cholesterol and total fat in pork.

5.11. Effect of dietary treatments on carcass characteristics

The effect of feeding experimental rations on carcass characteristics of pig is presented in Table 26 and 27. Six animals per each treatment were slaughtered at the end of finisher phase and the carcass characteristics were studied. No significant differences were found in any of the carcass characteristics among the treatments except in dressing percentage and hot carcass weight. The dressing percentage in pigs fed T4, T3 and T5 rations was significantly higher (P<0.01) than in those fed T1 and T2 rations. The hot carcass weight in pigs fed T4 rations was higher (P<0.05) than in T1, T2, T3 and T5 rations.

5.12. Cost of feed per kg pork production

The cost of feed per kg pork produced was lowest (P<0.01) in T4 fed pigs followed by T5, T3, T2 and T1 and it could be attributed to higher weight gains and superior feed conversion ratio.

5.13. Conclusions

The following conclusions were drawn from the present study:

i. The results of the current study indicated that SSM owing to its chitosan content can be an effective alternative to the use of antibiotic growth promoters in pigs. The enhanced small intestinal morphology, reduction in fecal shedding of *E. coli and Salmonella*, as well as increase in apparent digestibility of nutrients, clearly demonstrate its prebiotic effect.

ii. The shrimp shell meal supplementation in pigs had beneficiary effect in reducing bad cholesterol and increasing good cholesterol content of serum.

iii. Inclusion of shrimp shell meal containing COS in the rations of pigs has positive affect in lowering the muscle cholesterol and total fat in pork. However, further study is needed to elucidate the mechanism by which COS improved the quality of meat.

iv. The results of the current study also indicated that the lymphocyte concentration was increased following shrimp shell meal feeding in pigs suggesting beneficial effects on the immune system.

v. The feed cost of pork production can be reduced by including shrimp shell meal in standard diets.

vi. Inclusion of shrimp shell meal at 1% during creep phase and at 5% during grower and finisher stages was found to be optimum.

CHAPTER - VI

SUMMARY

Modern pig production practices that are associated with regular use of antibiotics as growth promoters or to control diseases contribute to the spread of drug-resistant pathogens in both livestock and humans posing a significant public health threat. Moreover with the ban of antibiotic growth promoters by several countries, extensive research work has become a continuous process leading to the identification of many potential alternative growth promoters such as acidifiers, probiotics, prebiotics etc., to reduce or eliminate the use of antibiotics in animal feeds. In recent years, multiple reports have described the beneficial effects of dietary oligosaccharides such as COS by enhancing host health status, modulating the intestinal microflora, increasing the digestibility of major nutrients and improving the small intestine morphological structure.

Hence, the present research work was carried out to evaluate the effect of supplementation of shrimp shell meal containing chitooligosaccharides (COS) as a source of prebiotic on the growth performance, nutrient digestibility, small intestine morphology, gut pathogen load, serum biochemical profile, haematological parameters, muscle cholesterol and carcass characters.

In the present study the effect shrimp shell meal as a prebiotic was evaluated for its effect on growth performance of crossbred pigs during creep, grower and finisher phases. During creep phase, a total of 240 pre-weaned piglets (10 d) were assigned at random to 5 dietary treatments of a control diet (T_1), T_1 supplemented with antibiotic (T_2), while in treatments T3, T4, and T5 dried and autoclaved shrimp shell meal containing 15.5% chitosan was included at 0.5, 1.0 and 1.5% respectively, such that the rations contained 700, 1500 and 2300 mg

chitosan/kg. The experimental diets were formulated as per NRC, 1998 and the diets were isocaloric and isonitrogenous. The TA, EE, and CF were higher in experimental diets fed in T3 to T5 than in T1, T2 and T3 due to SSM inclusion. Each treatment contained six replicate pens with eight piglets per pen. The experiment lasted for 42 days, during creep phase. The body weight gain at weekly intervals and the daily creep feed consumed were recorded. At the end of study period two piglets per treatment were slaughtered to study the intestinal morphology using scanning electron microscopy and micrometry and also to study the gut pathogen load.

From 240 piglets at creep stage, 40 pigs were selected as and when they attained 15 kg body weight for growth studies during grower phase (15-35 kg) and were shifted to the corresponding diet during finisher phases (35-70 kg live weight). During these phases the standard ration (T1) was supplemented with chlorotetracyclin (T2) while dried autoclaved shrimp shell meal was included at 2.5 (T3), 5.0 (T4) and 7.5% (T5), as a source of chitosan. The body weight gain at weekly intervals and the daily feed consumed were recorded. Two pigs per treatment at the end of grower phase and 6 pigs per treatment at the end of finisher phase were slaughtered to study the small intestine morphological structures and gut pathogen load, hematological parameters and carcass traits. A metabolism trial was conducted during finisher phase to study nutrient digestibility and nitrogen balance.

In all the phases, inclusion of SSM in diets T3 to T5 resulted in an increase in the villi height and decrease in the crypt depth leading to higher villi height to crypt depth ratio in the duodenum, jejunum and ileum segments of small intestine

and the diets could be ranked as T4>T2>T3>T5>T1 for their beneficial effects on small intestine morphological feachers.

Feeding diets containing antibiotic and shrimp shell meal reduced (P<0.01) the *E. Coli* and *Salmonella* count compared to control group, clearly demonstrating that SSM in T3 to T5 diets acted as a prebiotic by significantly (P<0.01) reducing *E. Coli* and *Salmonella* count in large intestinal content.

A metabolism trial was conducted during finisher phase. The digestibility of major nutrients except ether extract was increased when pigs were offered experimental diets containing shrimp shell meal. The digestibility of DM of in pigs fed T4 and T5 rations was significantly higher (P<0.05) than in those fed T1, T2 and T3 rations. The CP, CF and NFE digestibility was significantly higher (P<0.01) in T4 fed pigs than in other treatments while the digestibility of EE was significantly lower (P<0.01) in pigs fed T4 and T5 rations than those fed T1, T2 and T3 rations.

The nitrogen intake (g/d) was higher (P<0.01) in pigs fed T1 than in other treatments and was lowest in pigs fed T4 and it might be due to differences in feed intake during metabolism trail. Total nitrogen (g/d) loss was the highest (P<0.01) in T1 fed pigs than in T2, T3, T4 and T5 fed pigs and it could be attributed to the higher CP digestibility in pigs fed experimental diets than control diets. The nitrogen retention (g/d) was the lowest (P<0.05) in pigs fed T1 than in the other experimental diets. The nitrogen retention (% absorbed) was comparable among the treatments as there was no significant difference among the treatments.

In the present study blood profiles were also studied since the blood profiles of animals reflect the physiological disposition of their nutrition according to their internal and external environments. The serum total protein , IgG and

lymphocyte concentration increased (P<0.01) in response to feeding of SSM compared to control group.

There was a decrease (P<0.01) in serum total cholesterol, serum triglycerides, LDL cholesterol and increase in HDL cholesterol levels of pigs fed diets containing SSM (T3 to T5) than in T1 or T2 fed pigs. The better blood lipid profile led to a decrease in the muscle cholesterol and total fat.

No significant differences were found in any of the carcass characteristics among the treatments except in dressing percentage and hot carcass weight. The dressing percentage in pigs fed T4, T3 and T5 rations was significantly higher (P<0.01) than in those fed T1 and T2 rations. The hot carcass weight in pigs fed T4 diet was heiger (P<0.05) than in T1, T2, T3 and T5 diets. The cost of feed per kg pork produced was the least (P<0.01) in T4 fed pigs followed by T5, T3, T2 and T1 and it could be attributed to higher weight gains and superior feed conversion ratio.

The following conclusions were drawn from the present study:

i. The results of the current study indicated that SSM owing to its chitosan content can be an effective alternative to the use of antibiotic growth promoters in pigs. The enhanced small intestinal morphological feachers, reduction in fecal shedding of *E. coli and Salmonella,* as well as increase in apparent digestibility of nutrients, clearly demonstrate its prebiotic effect.

ii. The shrimp shell meal supplementation in pigs had beneficiary effect in reducing bad cholesterol and increasing good cholesterol content of serum.

iii. Inclusion of shrimp shell meal containing COS in the rations of pigs has positive affect in lowering the muscle cholesterol and total fat in pork.

However, further study is needed to elucidate the mechanism by which COS improved the quality of meat.

iv. The results of the current study also indicated that the lymphocyte concentration increased following shrimp shell meal feeding in pigs suggesting beneficial effects on the immune system.

v. The feed cost of pork production can be reduced by including shrimp shell meal in standard diets.

vi. Inclusion of shrimp shell meal at 1% during creep phase and at 5% during grower and finisher stages was found to be optimum.

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