

## **EFFECT OF SUPPLEMENTATION OF SHRIMP WASTE ON THE IMMUNE RESPONSE, HAEMATOLOGICAL CHARACTERS AND LIPID PROFILE IN CROSSBRED WEANED PIGLETS**

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**Abstract:** In a completely randomised design, the effect of antibiotic or chitosan from shrimp waste as prebiotic was evaluated using 36 weaned piglets (42 days). The standard grower ration (T1) was supplemented with chlorotetracyclin (T2) while shrimp shell meal was included at 2.5% (T3), 5.0% (T4) and 7.5% (T5), as a source of chitosan. The feed and water was made ad libitum. There was a significant increase ( $P < 0.01$ ) in serum total protein, primarily due to improvement in the globulin concentration, decrease ( $P < 0.01$ ) in serum total cholesterol, serum triglycerides, LDL Cholesterol and increase in HDL cholesterol due to shrimp waste supplementation and there was no significant difference among the pigs fed different treatments for the various carcass characters.

**Keywords:** Shrimp waste, Chitosan, Immune response, Lipid profile.

### **INTRODUCTION**

There is a dramatic change in the food habits of the people due to the increased trend towards urbanization and improving purchasing power led them to opt for nutritionally rich foods, such as animal protein foods. Pork is comparatively cheap source of animal protein of high biological value. Maintaining the demand for pork is mainly depends on the quality of the product which is largely affected by the feed that is fed to the animals. Modern pig production practices that are associated with regular use of antibiotics as growth promoters (Yang *et al.*, 2015) contributed to the spread of drug-resistant pathogens and posing a significant public health threat. Hence many alternative feeding strategies have been studied such as acidifiers, probiotics, prebiotics etc., to reduce the use of antibiotics in animal feeds. More recently dietary inclusion of chito-oligosaccharides (COS) in swine rations suggests that it provides a potential alternative to antibiotic supplements. Hence, a study was carried

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out to study the effects of dietary inclusion of shrimp shell meal containing chitosan on the immune response and lipid profile in cross breed pigs.

## **MATERIALS AND METHODS**

The feed ingredients like maize, soybean meal and de-oiled rice bran for preparation of standard experimental diets in ground form were procured locally and shrimp waste was procured from a shrimp processing factory in Nellore district of Andhra Pradesh. Representative samples of feed ingredients and creep feed were analyzed for proximate composition (AOAC, 2005). Fresh shrimp waste meal contained 30 % DM. Hence, it was sundried for 3 days to about 90% DM and subjected to grinding in a hammer mill and sterilized in an autoclave for 10 min at 121 °C and 15psi pressure. The dry matter content of fresh shrimp 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.

Thirty six weaned piglets (42 days) were assigned at random to 5 dietary treatments and fed grower ration. The standard ration (T1) was supplemented with chlorotetracyclin (T2) while shrimp waste was included at 2.5 (T3), 5.0 (T4) and 7.5% (T5), as a source of chitosan. Two pigs per treatment at the end of grower phase were slaughtered to study the parameters.

Blood samples were collected at the time of slaughter into vacuum tubes containing no additive and tubes containing K<sub>3</sub> 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. The serum was separated by centrifugation for 30 min at 2000 x g at 4°C and the aliquot was stored at -4°C for determination of serum profiles.

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. The LDL cholesterol is calculated by the difference between total cholesterol and HDL cholesterol. Serum total proteins and albumin were estimated by using diagnostic kit (Monozyme India Limited) and globulin was estimated by subtracting albumin from total protein. The muscle sample from each slaughtered animal was collected and preserved at -20 °C for estimation of muscle cholesterol and triglyceride content. Lipids from muscle tissue were extracted according to the procedure of Folch *et al.* (1957). The total cholesterol was estimated by enzymatic method using standard kits. . The data obtained by slaughter of two pigs at the end of grower phase were subjected to one way ANOVA from summary data.

## RESULTS AND DISCUSSION

The blood profiles of animals reflect the physiological disposition of their nutrition according to their internal and external environments. Therefore, we measured these characteristics to determine the response by which COS influenced. In the present study, serum total protein concentration was increased ( $P < 0.01$ ) in response to COS supplementation compared to control group (Table 1) 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 is increase in the serum IgG concentration which are in agreement with Wang *et al.*, (2009). This response indicates that COS supplementation had beneficial effects on the immune system. In addition, the lymphocyte concentration was increased ( $P < 0.01$ ) following COS supplementation which may indicate that COS also had beneficial effects on the immune system in agreement with Eze *et al.* (2010) and Yan and Kim (2011).

There was a decrease ( $P < 0.01$ ) in serum total cholesterol, serum triglycerides, LDL cholesterol and increased HDL cholesterol levels (Table 2) 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 which are in agreement with Tang *et al.*, (2005); Zhang *et al.*, (2012) and Gronde *et al.*, (2016).

Ma *et al.*, 2001 and Tang *et al.*, 2005 reported that the mechanism in reduced triglycerides (TG) and total cholesterol in serum was 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 faeces, thus the absorption of fat and cholesterol is decreased and the same mechanism for the decreased levels of triglycerides and cholesterol.

Gronde *et al.*, 2016 reported that chitosan acts 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 (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.

The effect of supplementation of shrimp shell meal containing chitosan on muscle cholesterol and total fat can be compared with studies of Kim *et al.* (2008) where in they reported addition of COS in the diets of finishing pigs reduced the cholesterol in pork without affecting its quality. In the present study, in treatments T3 to T5 containing COS, there was decrease ( $P<0.01$ ) in muscle cholesterol and total fat in finisher pigs (Table 4) and the same trend was also observed in pigs fed grower rations.

## CONCLUSION

It was concluded that shrimp waste meal at 5% level in crossbred pig diets was beneficial as alternative to antibiotic feed additives and observed that COS alter the serum lipid profile by decreasing the bad cholesterol, total cholesterol or serum triglycerides and by increasing the good cholesterol.

**Table 1: Effect of dietary treatments on haematological parameters in weaned piglets**

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

<sup>abc</sup> values in a row not sharing common superscripts differ significantly \*\*( $P<0.01$ )

**Table 2: Effect of treatments on serum lipid (mg/dl) profile in weaned piglets**

Parameter	T1	T2	T3	T4	T5
Total cholesterol**	76.75 <sup>a</sup> ±0.25	72.00 <sup>b</sup> ±0.50	71.75 <sup>b</sup> ±0.25	64.75 <sup>c</sup> ±0.25	69.00 <sup>c</sup> ±0.50
Triglycerides**	72.25 <sup>a</sup> ±0.75	68.00 <sup>b</sup> ±0.50	68.25 <sup>b</sup> ±0.25	60.25 <sup>c</sup> ±0.75	68.00 <sup>b</sup> ±0.50
HDL <sup>NS</sup>	41.50±0.50	43.25±0.25	43.75±0.25	44.25±0.75	44.25±0.75
LDL**	35.25 <sup>a</sup> ±0.25	28.75 <sup>b</sup> ±0.25	28.00 <sup>b</sup> ±0.50	20.50 <sup>d</sup> ±1.00	24.75 <sup>c</sup> ±0.25

<sup>abc</sup>values in a row not sharing common superscripts differ significantly \*\*( $P<0.01$ ); \*( $P,0.05$ )

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