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Effect of Feeding Fish Waste Silage Meal on the Immune Status of Piglets

Nithya G.[1], Suresh R.[2*] and Sumitha P.[3]
[1]MVSc Scholar, Department of Animal Nutrition, TANUVAS-VCRI, Orathanadu (Tamil Nadu), India.
[2]Assistant Professor, Department of Animal Nutrition, TANUVAS-VCRI, Salem (Tamil Nadu), India.
[3]Assistant Professor, TANUVAS-VCRI, Orathanadu (Tamil Nadu), India.

(Corresponding author: Suresh R.)*
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ABSTRACT: A study was conducted on twelve healthy newborn Large White Yorkshire piglets from two Dams, for three months to assess the effect of feeding fish waste silage meal (FWSM) on the immune response of the animals. These piglets were divided into two groups, namely the T_0 (Control) and T_1 (Treatment) group. During the creep phase, the control group (T_0) was fed a soybean meal-based ration and the treatment group (T_1) was fed with FWSM replacing part of the soybean meal-containing ration, while in the grower phase, both groups were fed soybean meal based ration without FWSM supplementation. At the end of the trial, cell-mediated and humoral immunity was assessed by administering PHA-p mitogen and 20% sheep RBC's, respectively. The results showed a significant difference in cell-mediated immunity, whereas no significant difference was observed in HI response, but compared to the control (T_0) group better HI response was noticed in the supplemented (T_1) group.

Keywords: Fish waste, silage, immunity, PHA-p, Sheep RBC.

INTRODUCTION

The scarcity of protein is a primary constraint in animal farming, primarily due to its higher cost and restricted availability (Ozyurt *et al.,* 2017). Enormous amounts of solid wastes were discarded by the fish processing plants and markets which can account for up to 50-80% of the initial raw material (Mohanty *et al.,* 2018). Fish silage is a liquified product made from whole fish or parts of fish through acid preservation (Tatterson and Windsor 1974), fermentation of lactic acid bacteria, or both (Raa *et al.,* 1982), which improves the action of endogenous enzymes in the fish to break down tissues and limits the growth of pathogenic bacteria. Hauzoukim *et al.* (2021) also mentioned that animal protein sources have advantages over vegetable proteins with balanced amino acids and minimal concentrations of anti-nutritional factors which have the potential to interfere with the digestion of other nutrients.

Through the process of ensiling, the fish waste can be transformed into a hydrolyzed mixture of proteins, lipids, minerals and other nutrients that are readily digestible and absorbable by livestock (Abdallah *et al.,* 2018). Peptides and free amino acids generated through protein hydrolysis may serve as stimulants for non-specific immunity (Goosen *et al.,* 2016). Among various dietary proteins, fish proteins are expected to contain a higher amount of bioactive sequences, presenting a potential source for potent immune-modulating agents (Duarte *et al.,* 2006). There is limited information available regarding the immune response of animals fed with silage meal derived from fish waste.

MATERIALS AND METHODS

An experiment was carried out for a period of 90 days in Large White Yorkshire piglets. The piglets were allowed to suckle sow milk for 2 weeks, from the age of14 days to 56 days (weaning) the treatment group piglets were fed with 10% FWSM supplemented feed, the control group piglets were fed with conventional soybean meal (SBM) based ration, from 56 to 90 days both T_0 and T_1 group piglets were fed with SBM based ration (0% FWSM).

Experimental animals. Twelve Large White Yorkshire piglets were selected from the piggery unit, Livestock Farm Complex, Veterinary College and Research Institute, Orathanadu. All the animals were vaccinated against Classic Swine Fever (CSF) at the age of 50 days and were dewormed at the time of weaning. The piglets were divided into two groups as uniformly as possible concerning sex and weight.

Housing and management. In the creep phase, piglets were kept along with their dam in individual pens. In the grower phase, the control and treatment group piglets were grouped separately. Throughout the study period, the animals were maintained hygienically and provided with pre-weighed wet mash feed twice a day and adlibitum of clean potable drinking water.

Experimental ration. The rations were prepared as per NRC, 1998 feeding standard. In the creep phase, the control group (T_0) received a ration with soybean meal as the primary protein source, while the treatment group (T_1) had soybean meal partially substituted with FWSM and in the grower phase both the groups were provided with soybean meal as a protein source. All diets were

adjusted to be iso-caloric and iso-nitrogenous, ensuring a consistent level of energy and protein across the experimental groups. The composition of the rations is given in Table 1.

Immune response study. The effect of FWSM supplementation on cell-mediated immune response was assessed by delayed-type hypersensitivity (DTH) with response to Phytohaemagglutinin-p (PHA-p) mitogen (Kim *et al.,* 2000). About 0.1ml of PHA-p mitogen was injected intra-dermally on the left flank and 0.1 ml of normal saline was injected on the right flank asa negative control. The thickness of the skin was measured at 0, 6, 12, 24 and 36 hours of post-inoculation and the difference in skin thickness of PHA-p and normal saline injection site (in mm) was calculated and expressed as CMI response.

The humoral immune response was assessed by antibody response to the sheep red blood cells (SRBC's) as described by Wagmann and Smithies (1966). One ml of 20% SRBC suspension was injected intramuscularly into the piglets and blood samples were collected to assess the antibody titre. The serum samples separated from blood were subjected toa micro-haemagglutinationtest to assess the humoral immune response.

The CMI and HI response was assessed on six pigs from each dietary group on the 60th day post-weaning.

Blood collection and analyses. The sera samples for antibody determination were collected at 0, 14 and 21 days of post-administration of 20 percent SRBC's and stored at -20°C. Before performing the assay, sera samples were thawed and kept at 56°C for 30 min for inactivation. The antibody titre against SRBC was measured by a micro-titrehaemagglutination assay (Wagmann and Smithies 1966).

Statistical analysis. Each piglet was considered an experimental unit for data analysis. All the data obtained from the study were subjected to analysis of variance (ANOVA). The difference among the treatment groups was compared by Tukey's test and probability values $P<0.05$ were considered significant. All the data were analyzed using Statistical Package SPSS (Version 20.0).

RESULTS AND DISCUSSION

The results of Cell-Mediated Immunity (CMI), in terms of Delayed-Type Hypersensitivity (DTH) response to PHA-p, are outlined in Table 2 and illustrated in Fig. 1. A highly significant improvement ($P<0.01$) was observed in skin indurations, measured in terms of skin thickness (mm), among the dietary treatments. The supplementation of FWSM (T_1) demonstrated a notable increase in skin thickness (mm) compared to the control group (T_0) with the observed values of 4.22 for the control group (T_0) and 4.61 for the treatment group (T_1). Furthermore, the skin thickness increased and peaked at 24 hours, followed by a subsequent decline up to 36 hours post-inoculation in both dietary treatments and it was highly significant ($P<0.01$). The increase in the skin fold thickness within 12 hours after PHA-p injection, followed by a decrease after 24 hours is attributed to the PHA-p concentration and the peak thickness measured between 12 to 24 hours which concurred with the study conducted by Ekkel *et al*. (1995) on immune response in 24 weaned piglets by administering intradermal injections of Phytohemagglutinin (PHA-p) at seven different concentrations (1, 5, 10, 25, 50, 100 and 250 µg). Goosen *et al*. (2016) documented the phagocytic activity for low silage made with fresh rainbow trout viscera diet (160g/kg of diet) was higher than control (fish meal) and formic acid added diet, but the high silage (285g/kg of diet) did not differ from other treatment.

The results pertaining to humoral immunity (log_2 titre) of piglets fed with different dietary treatments were summarized in Table 3 and illustrated in Fig. 2. The humoral immunity was evaluated based on the antibody response to sheep erythrocytes (SRBC) using the Hemagglutination (HA) test. The antibody titer against SRBC reached its maximum on day 14 of post-inoculation, followed by a subsequent declining trend up to day 21 of post-inoculation and there was a significant difference noticed between the period ($P<0.01$).

Hessing *et al*. (1995) conducted a study on suckling piglets to determine humoral immunity with 10 mg of Bovine Serum Albumin (BSA) and they collected blood on 7, 14, 21 and 28 days post administration of BSA and subjected to ELISA. They observed increased antibody response on day 14 while peak response was observed on day 21, which was similar to the findings of this study.

A study conducted by Buschmann *et al.* (1974) in pigs using SRBC, revealed both hemagglutinating and hemolyzing antibody responses against SRBC were found in both immunized and non-immunized pigs. He also added that the immune response in pigs immunized with a constant dose of sheep erythrocytes as determined by the occurrence of plaque-forming cells in the spleen and serum antibodies varies with the breed and season.

A significant increase in phagocytosis noticed in the peritoneal macrophages and IgA cells of the small intestine, IL-4+, IL-10+ and IL-6+ cells was observed only after 7 days of Fish Protein Concentrate administration. For IFNγ+ and TNFα+ cells, a significant increase was observed after both 5 and 7 days of FPC feeding.

Among various dietary proteins, fish proteins are expected to contain a higher amount of bioactive sequences, presenting a potential source for potent immune-modulating agents (Duarte *et al.,* 2006).

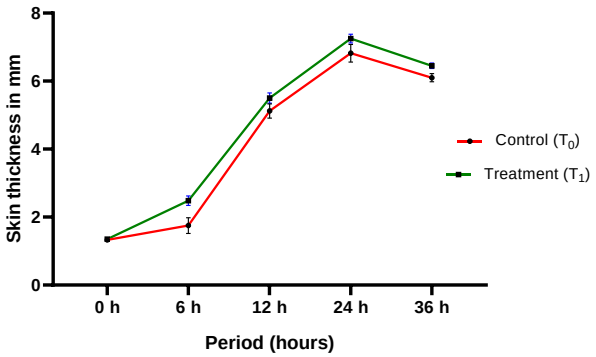

Fig. 1. Effect of FWSM supplementation on DTH response (skin thickness in mm) against PHA-p.

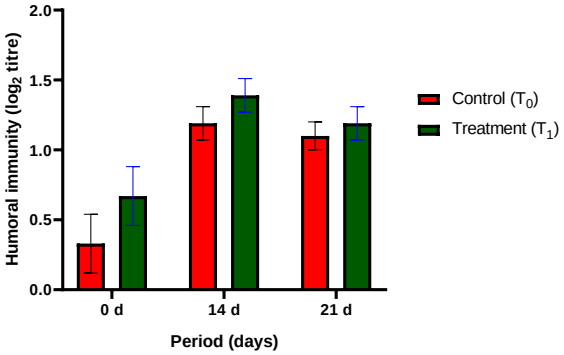

Fig. 2. Effect of FWSM on humoral immunity (log_2 titre) in pigs measured as antibody response to sheep RBC.

Table 1: Composition and nutritive value of the experimental ration fed to piglets.

Ingredient (%)	Control ration (T_0)		Treatment ration (T_1)		Both Control and Treatment ration (T_0 & T_1)
	3-5 kg Bwt	5-10 kg Bwt	3-5 kg Bwt	5-10 kg Bwt	10-20 kg Bwt
Crushed maize	46.50	49.20	41.25	44.76	52.71
Fish Waste Silage Meal (FWSM)	0.00	0.00	10.00	10.00	0.0
Soybean meal	48.78	44.48	44.50	38.50	36.50
De-oiled rice bran	0.00	1.30	0.11	2.50	4.20
Oil/fat	0.70	1.00	0.70	0.70	2.0
Mineral mixture	2.00	2.00	2.00	2.00	1.90
Methionine	0.10	0.05	0.01	0.00	0.02
Lysine	0.10	0.05	0.01	0.01	0.05
Salt	0.50	0.50	0.50	0.50	0.50
Hyblend AB_2D_3K	0.01	0.01	0.01	0.02	0.10
US Curatox	0.05	0.05	0.05	0.05	0.07
Ultra TM	0.10	0.10	0.10	0.10	0.10
Tefroli plus	0.05	0.05	0.05	0.05	0.10
Multiplex Ds	0.01	0.01	0.01	0.01	0.05
Ultravite M	0.50	0.50	0.50	0.50	0.70
Calcite	0.60	0.70	0.20	0.30	0.60
Total	**100**	**100**	**100**	**100**	**100**
Nutrient composition (As fed basis)					
Digestible energy (kcal/kg)*	**3397.5**	**3398.5**	**3375.8**	**3403.8**	**3408.8**
Crude Protein (%)*	**25.2**	**23.7**	**25.7**	**23.7**	**20.98**

*Calculated value as fed basis

Table 2: Effect of feeding Fish Waste Silage Meal (FWSM) on cell-mediated immunity of piglets measured as DTH response (skin thickness in mm) against PHA-p (n=6).

Treatment	Period (hours)					Treatment mean	SEM	Significance		
	0	6	12	24	36			T	P	T*P
Control (T_0)	1.33 ± 0.04	1.75 ± 0.23	5.12 ± 0.21	6.82 ± 0.26	6.10 ± 0.22	4.22 ± 0.43^{B}	0.05	0.00^{**}	0.00**	0.35
Treatment (T_1)	1.35 ± 0.04	2.48 ± 0.14	5.50 ± 0.15	7.25 ± 0.13	6.45 ± 0.08	4.61 ± 0.43^{A}				
Period mean	1.34 ± 0.03^{Z}	2.12 ± 0.17^{Y}	5.31 ± 0.14^{X}	7.03 ± 0.15^{V}	6.28 ± 0.12^{W}					

** P values bearing superscripts differ more significantly (P<0.01)
AB/UVXYZ Means with different superscripts within a column (AB) or row (VWXYZ) differ significantly

Table 3: Effect of feeding Fish Waste Silage Meal (FWSM) on humoral immunity (log_2 titre) in piglets measured as antibody response to sheep RBC (n=6).

Treatment	Period (days)			Treatment mean	SEM	Significance		
	0	14	21			T	P	T*P
Control (T_0)	0.33 ± 0.21	1.19 ± 0.12	1.10 ± 0.10	0.88 ± 0.13	0.06	0.11	0.00**	0.75
Treatment (T_1)	0.67 ± 0.21	1.39 ± 0.12	1.19 ± 0.12	1.08 ± 0.11				
Period mean	0.50 ± 0.15^Z	1.29 ± 0.09^Y	1.15 ± 0.08^Y					

** P values bearing superscripts differ more significantly (P<0.01)
YZ Means with different superscripts within a row (YZ) differ significantly

CONCLUSIONS

The supplementation of FWSM had a significant improvement in CMI, however, no significant difference was observed in HI response, but a better HI response was noticed in the supplemented group. Improved immune status of piglets is an indicator of health status, good health status is vital for reducing disease occurrence and productivity. Based on the results, 10 percent FWSM supplementation during the creep phase positively impacts piglets immunity without any adverse effects.

FUTURE SCOPE

Fish Waste Silage Meal can be tried.

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Conflict of Interest. None.

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