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Folic Acid Supplementation on Piglets Immunity

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ABSTRACT: This experiment was conducted to study the effect of dietary folic acid supplementation on cell mediated (CM) and humoral immune (HI) response in growing piglets born to sow fed diet with or without supplemental folic acid during gestation and lactation. After weaning, eighteen piglets (Landlly) from each group (T_0, T_1 and T_2) were selected and randomly sub-divided into 3 sub-groups of 6 each in an experiment based on 3×3 factorial design. The treatments were; D_0O_0, D_0O_1, D_0O_2, D_1O_0, D_1O_1, D_1O_2, D_2O_0, D_2O_1 and D_2O_2, where, D_0: Dam with no supplementation of folic acid (FA); D_1: Dam with FA supplementation during gestation (@15 mg/kg of feed); D_2: Dam with FA supplementation during gestation and lactation both (@15 mg/kg of feed); O_0: Offspring with no supplementation of FA; O_1: Offspring with FA supplementation @ 2.5 mg/kg of feed and O_2: Offspring with FA supplementation @ 5.0 mg/kg of feed. The CMI and HI response was assessed 150 days of post-weaning. The cell-mediated and humoral immunity were significantly higher ($P<0.01$) in folic acid supplemented groups in comparison to control (D_0O_0) group. The humoral immunity (HI) response, was better ($P<0.01$) in D_0O_2, D_1O_2, D_2O_1 and D_2O_2 groups when compared to D_0O_0. Thus, based on the results concerning to CMI and HI response it is evident that postnatal folic acid supplementation at 2.5 or 5.0 mg/kg feed is beneficial in terms of better immunity.

Keywords: Cell mediated, Humoral, Immune, Piglets.

INTRODUCTION

Folic acid and vitamin B_{12} play a vital role in the healthy balance of the immune system (Mikkelsen and Apostolopoulos 2019). Deficiency or inadequate level of these vitamins can drastically alter immune responses by affecting the production of DNA, RNA, protein synthesis, inhibiting the activity of immune cells and interfering with metabolic processes (serine, glycine and purine cycles) and methylation. Low serine can also interfere with the immune system function by preventing proper antibody formation and interfering with the proper functioning of effect or T cells (Ma *et al.*, 2017; Mahmood, 2014). In swine production, supra-nutritional folic acid supplementation is essential to overcome the higher HCY levels and IUGR. Although pigs cannot synthesize folic acid, bacteria in their lower gut can synthesize and make the faeces as another source of the vitamin (Abad and Gregory 1987). Green leafy plants are a promising and feasible source of folic acid (Gorelova *et al.*, 2017). Change in rearing and waste removal systems have reduced the sow's access to leafy plants and faecal material. These modifications in rearing system lead to inaccessibility of two cheaper sources of folic acid for pigs, ushering researchers to reevaluate the management systems to balance folic acid in swine diets.

MATERIALS AND METHODS

After weaning, eighteen piglets (Landlly) from each group (T_0, T_1 and T_2) born to sow fed diet with or without supplemental folic acid during gestation and lactation were selected from the Piggery farm, LPM section of the institute and ear-tagged and randomly sub-divided into 3 sub-groups of 6 each in an experiment based on 3×3 factorial design. The duration of the study was from the day of weaning to 150 days of post-weaning. The treatments were D_0O_0, D_0O_1, D_0O_2, D_1O_0, D_1O_1, D_1O_2, D_2O_0, D_2O_1 and D_2O_2, where,

D_0 : Dam with no super- supplementation of folic acid (FA)

D_1 : Dam with FA supplementation during gestation (@15 mg/kg of feed)

D_2 : Dam with FA supplementation during gestation and also during lactation (@15 mg/kg of feed)

O_0 : Offspring with no super- supplementation of FA

O_1 : Offspring with FA supplementation @ 2.5 mg/kg of feed

O_2: Offspring with FA supplementation @ 5.0 mg/kg of feed

The basal diet (mash feed) was prepared as per NRC (1998). The post weaned animals were offered weighed quantity of a mash as a single meal at 09:30 AM to meet their requirements (NRC, 1998) along with free access to clean drinking water. Piglets in the O_0 group were fed with only basal diet. Whereas, piglets in O_1 and O_2 groups were fed 2.5 mg/kg and 5 mg/kg folic acid (MB Vet Chem, Navi Mumbai, India) supplemented diet, respectively. The physical composition of the experimental ration is presented in below

Physical composition of basal diets (as-fed basis) for piglets.

Ingredients (%)	Body weights (kg)			
	5-10	10-20	20-50	50-80
Crushed maize	54.6	61.3	66.5	75.0
De-oiled soybean meal	41.6	33.9	27.4	19.3
Wheat bran	0.8	2.0	4.0	3.80
Calcite	0.2	0.1	0.2	0
Dicalcium Phosphate	0.6	0.5	0	0
Mineral and vitamin mixture*	1.5	1.5	1.5	1.5
L-Lysine	0.2	0.1	0	0
DL-Methionine	0.1	0.2	0	0
Sodium chloride	0.4	0.4	0.4	0.4
Folic acid (mg/kg diet)	0.3	0.3	0.3	0.3
Crude protein (%) **	23.6	20.8	18.4	15.5
Digestible energy (Kcal/Kg) **	3396	3392	3397	3409
Lysine (%) **	1.27	1.02	0.80	0.62
Methionine (%) **	0.40	0.47	0.25	0.22
Calcium (%) **	0.78	0.70	0.60	0.50
Total phosphorus (%) **	0.64	0.61	0.52	0.49

*Each 1kg contains: vitamin A 20, 00,000 IU; vitamin D_3 4, 00,000 IU; vitamin B_2 0.8 g; vitamin E 0.3 g; vitamin K 0.4 g; vitamin B_{12} 2.4 mg; calcium pantothenate 0.1 mg; niacin 4 g; choline chloride 60 g; calcium 0.28 g; manganese 11 g; iodine 0.4 g; iron 3 g; zinc 6 g; copper 0.8 g; cobalt 0.18 g; phosphorus 80 g.
**Calculated values as fed basis.

A. Immune response study

The effect of dietary folic acid supplementation on humoral immune response was studied as per the micro-haemagglutination assay described by Wagmann and Smithies (1966). The cell-mediated immune response was assessed as per the method described by Kim *et al.* (2000) through in vivo sub-cutaneous delayed-type hypersensitivity (DTH) response by injecting phytohaemagglutinin-P (PHA-P) intradermally in the flank region. After intradermal injection, the thickness of the skin was measured at 6, 12, 24 and 36 hours. Finally, the difference in skin thickness of PHA-P and normal saline injection site in millimeter was calculated and expressed as CMI response. CMI and HI response study was performed on six pigs from each dietary group on 150 days of post-weaning.

B. Blood collection and analyses

The serum samples for antibody determination were collected at 0, 14 and 21-day 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 micro-titre haemagglutination assay (Wagmann and Smithies 1966).

C. Statistical analysis

Each piglet was considered as 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 data of CMI in terms of DTH response to PHA-p are presented in Table 1 and depicted in Fig. 1. The findings concerning to cell-mediated immunity (CMI) in terms of DTH response to PHA-p showed a significant ($P<0.01$) improvement in the skin indurations in terms of skin thickness (mm) among the dietary treatments. As illustrated in Fig. 1, the skin thickness (mm) increased and was at peak at 24h, afterwards there was a decline up to 36 h of post-inoculation in all the dietary groups. There was a significant ($P<0.01$) TxP interaction as the response was more pronounced at 24 h post-injection. All the treated groups exhibited significantly higher CMI response in comparison to control (D_0O_0) except D_1O_0 group, however, CMI response was highest in D_2O_1 and D_2O_2 groups indicating that supplementation of sows both during gestation and lactation along with post weaned piglets is more effective. Gross *et al.* (1975) reported that depressed CMI response in megaloblastic anemia (folic acid deficiency) was reversed by folate supplementation. Further, a severe defect in the antibody response was observed in folate deficient white rats (Axelrod, 1971).
The data pertaining to humoral immunity (log2 titre) of pigs fed various dietary treatments are presented in Table 2 and an illustration (Fig. 2). The humoral immunity was expressed as the antibody response to sheep erythrocytes (SRBC) deploying HA test. The humoral immunity (HI) as assessed by antibody response to sheep erythrocytes (SRBC) showed significantly ($P<0.01$) higher antibody titre in D_0O_2, D_1O_2, D_2O_1 and D_2O_2 groups when compared to D_0O_0

where there was no supplementation at any stage. In all the groups the peak of antibody titre was at 14 d post-injection thereafter came down at 21 day, which is a normal pattern of antibody titre. This corroborates well with the findings of Grieshop *et al.* (1999) which indicates that piglets supplemented with folic acid and born from sows supplemented with folic acid during gestation or gestation plus lactation periods exhibited a greater (P<0.05) secondary antibody response to SRBC's. A similar finding was observed by Ezzat *et al.* (2011) in Matrouh poultry strain supplemented with folic acid. Li *et al.* (2016) conducted an experiment in broilers to investigate the effect of *in ovo* injection of folic acid on folate metabolism, immune function and the involved epigenetic modification. The study revealed that increasing level of folic acid supplementation improved the IgG, IgM and plasma lysozyme activity in broilers. Further, the spleenic expression level of immune-related IL-2 and IL-4 genes were up-regulated, whereas, IL-6 was down-regulated, in the folic acid (100 and 150 µg) supplemented groups.

Table 1: Effect of folic acid (FA) supplementation on cell-mediated immunity of pigs measured as DTH response (skin thickness in mm) against PHA-p

Treatment†	Period (hrs)				TreatmentMean	SEM	Significance		
	0 h	12 h	24 h	36 h			T	P	T*P
D_0O_0	2.09^{n}±0.04	5.29^{kl}±0.19	8.13^{f}±0.08	4.02^{m}±0.15	4.88^{F}±0.46	0.028	<0.001	<0.001	<0.001
D_0O_1	2.11^{n}±0.07	5.71^{jk}±0.13	8.37^{f}±0.10	5.90^{jk}±0.12	5.52^{DE}±0.47				
D_0O_2	2.12^{n}±0.05	6.03^{jk}±0.18	8.58^{def}±0.13	6.16^{hijk}±0.05	5.72^{D}±0.49				
D_1O_0	2.10^{n}±0.05	5.74^{jk}±0.06	8.44^{ef}±0.19	4.44^{lm}±0.13	5.18^{EF}±0.48				
D_1O_1	2.15^{n}±0.09	6.12^{ijk}±0.16	9.30^{bcde}±0.33	7.97^{fg}±0.17	6.38^{C}±0.57				
D_1O_2	2.17^{n}±0.13	6.98^{hi}±0.26	10.17^{ab}±0.28	8.72^{cdef}±0.17	7.01^{B}±0.64				
D_2O_0	2.13^{n}±0.08	6.03^{jk}±0.06	8.54^{def}±0.12	5.71^{jk}±0.20	5.60^{D}±0.48				
D_2O_1	2.16^{n}±0.07	6.49^{hij}±0.10	10.16^{ab}±0.31	9.38^{bcd}±0.34	7.05^{B}±0.66				
D_2O_2	2.17^{n}±0.06	7.06^{gh}±0.04	11.05^{a}±0.23	9.53^{bc}±0.21	7.45^{A}±0.71				
Period mean	2.13^{Z}±0.02	6.16^{Y}±0.09	9.19^{W}±0.15	6.87^{X}±0.28					

†D_0O_0: No supplementary folic acid (FA) to the offspring born to dam receiving no supplement during gestation
D_0O_1: FA was supplemented at 2.5 mg/kg to the offspring born to dam receiving no supplement during gestation
D_0O_2: FA was supplemented at 5 mg/kg to the offspring born to dam receiving no supplement during gestation
D_1O_0: No supplementary FA to offspring born to dam receiving supplementary FA during gestation
D_1O_1: FA was supplemented at 2.5 mg/kg to the offspring born to dam receiving supplementary FA during gestation
D_1O_2: FA was supplemented at 5.0 mg/kg to the offspring born to dam receiving supplementary FA during gestation
D_2O_0: No supplementary FA to offspring born to dam receiving supplementary FA during gestation and lactation
D_2O_1: FA was supplemented at 2.5 mg/kg to the offspring born to dam receiving supplementary FA during gestation and lactation
D_2O_2: FA was supplemented at 5.0 mg/kg to the offspring born to dam receiving supplementary FA during gestation and lactation
^{abcdefghijklmn} Means bearing different superscripts differs significantly (P≤0.05) and (P≤0.01)
$^{ABCDEF/WXYZ}$Means bearing different superscripts within a column (ABCDEF) or row (WXYZ) differs significantly

Table 2 Effect of folic acid (FA) supplementation on humoral immunity (log_2titre) in pigs measured as antibody response to sheep RBC.

Treatment†	Period (days)			Treatment Mean	SEM	Significance		
	0-d	14-d	21-d			T	P	T*P
D_0O_0	1.25±0.13	1.58±0.19	1.42±0.15	1.42^{E}±0.09	0.032	<0.001	0.001	0.999
D_0O_1	1.58±0.15	1.75±0.25	1.67±0.14	1.67^{CDE}±0.11				
D_0O_2	1.83±0.11	2.08±0.08	1.83±0.11	1.92^{BCD}±0.06				
D_1O_0	1.33±0.14	1.58±0.15	1.42±0.15	1.44^{E}±0.08				
D_1O_1	1.67±0.19	1.83±0.24	1.75±0.18	1.75^{BCDE}±0.12				
D_1O_2	1.92±0.15	2.17±0.24	1.83±0.21	1.97^{ABC}±0.12				
D_2O_0	1.42±0.15	1.67±0.19	1.42±0.15	1.50^{DE}±0.09				
D_2O_1	1.83±0.17	2.42±0.19	2.17±0.17	2.14^{AB}±0.11				
D_2O_2	2.25±0.13	2.58±0.15	2.25±0.13	2.36^{A}±0.08				
Period mean	1.68^{Y}±0.06	1.96^{X}±0.07	1.75^{Y}±0.06					

^{abcde} Means bearing different superscripts differs significantly (P≤0.05) and (P≤0.01)
$^{ABCDE/XY}$Means bearing different superscripts within a column (ABCDEF) or row (XY) differs significantly

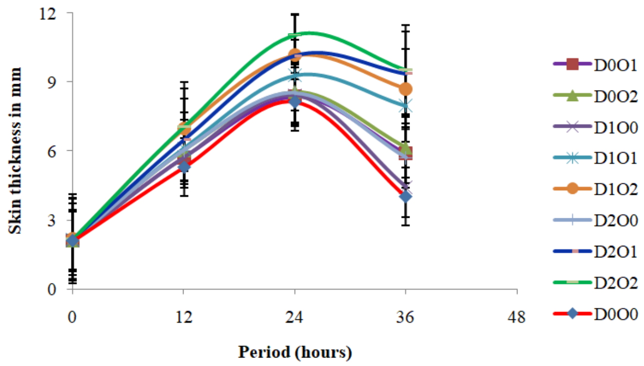

Fig. 1. Effect of folic acid supplementation on DTH response (skin thickness in mm) against PHA-p.

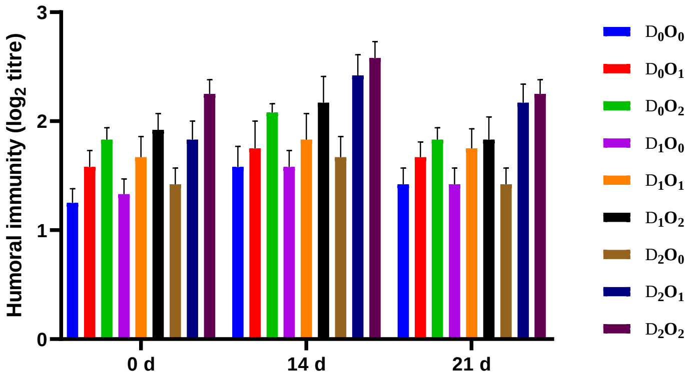

Fig. 2. Effect of folic acid supplementation on humoral immunity (log_2 titre) in pigs measured as antibody response to sheep RBC.

CONCLUSIONS

Based on the study results, it could be concluded that post-natal folic acid supplementation @ 2.5 and 5.0 mg/kg diet improved the immunity of piglets born from dam which had dietary supplementation of folic acid (@ 15 mg/kg feed) both during gestation and lactation. Hence, based on the results evinced in this study, for an improved and economical productivity in piggery, folic acid supplementation both at gestational and lactational stage for sows (@ 15 mg/kg feed) and for progeny folic acid supplementation (@ 2.5 mg/kg diet) may be used as potential feed additive for grower-finisher pigs.

FUTURE SCOPE

Folic acid and cyanocobalamin combination can be tried.

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Conflict of Interest. None.

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