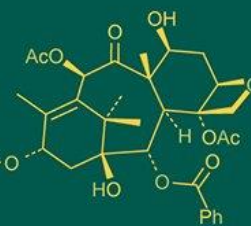
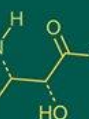
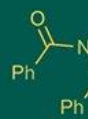


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Haematological responses of suckling piglets to combined multi-strain probiotic and multi-mineral supplementation

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Abstract

The purpose of the current study was to evaluate the impact of early life multi-strain probiotic (MSP) supplementation and mineralisation on haematology. For the first seven days of life, eight piglet litters from sows with comparable parity were randomly assigned to four groups and given the following treatments: control (normal saline), multimineral (MM), multi-strain probiotic, and multimineral + multi-strain probiotic. In conclusion, the mean Haemoglobin, packed cell volume and total erythrocyte count was higher in multimineral (MM), multi-strain probiotic, and multimineral + multi-strain probiotic group as compare to control group whereas the mean total leucocyte count and neutrophil count on day 14 to 21 and lymphocyte count on day 21 were significantly ($p < 0.05$) lower from day 0 value in multi-strain probiotic group.

Keywords: Multi-strain probiotics, multimineral, haematology, piglets

Introduction

Piglet diarrhoea is a recurring issue in swine farms and a major contributor to increased morbidity and death in new-born pigs. (Zhou *et al.*, 2023) [24]. In pig farming, diarrhoea within the first seven days of life causes subpar performance and substantial financial loss. (Farmer and Edwards, 2022) [6]. Piglets must have a healthy gut microbiota and an intact gut barrier in their early years in order to be protected against intestinal illnesses. New-born piglets' gut is usually thought to be sterile, but because of their early exposure to the outside world, nursing piglets are susceptible to colonisation by dangerous infections, which increases the risk of diarrhoea. (Wang *et al.*, 2021) [20]. New-born piglets' survival depends on their mother's passive immunity, which consists of three elements at minimum: There are three main forms of humoral immunity: systemic humoral immunity, local humoral immunity and maternal immunocompetent cells that transmit cellular immunity through mammary secretions (Salmon, 2000) [16]. Previous research has shown that mammary gland secretions, which are mostly colostrum, contain live immune cells in addition to antibodies. These cells are mainly lymphocytes, macrophages, neutrophils, and epithelial cells. Colostrum have the ability to transfer both humoral and cell-mediated immunity, according to Nechvatalova *et al.* (2011) [13]. The bulk of leukocytes in the blood of new-born pigs are colony-stimulating leukocytes, which are absorbed via the GI tract and affect the immune system of the piglets.

We hypothesised that MSP supplementation and mineralization in early life of piglets would affect the haemoglobin and total erythrocyte count positively. The aim of the present study was to evaluate the effect of co administration of MSP and multi-minerals to piglets in early life on hemogram until weaning.

Materials and Methods

Multi-strain probiotics and multi-minerals

The probiotic strains: the mixture of MSP, consist of *Limosilactobacillus reuteri* MW 367846.1, *Limosilactobacillus reuteri* KF149014.1, *Pediococcus pentasaceus* MK611260.1 and *Limosilactobacillus johnsoni* HM772969, *Pediococcus acidilactici* KU837245.1 was obtained from Animal Nutrition Division of the institute.

Cross streaking was done to check synergistic activity of all strains on MRS agar. Multi-minerals (MM) in injectable form (Stimvet, each mL contained 5 mg selenium, 40 mg zinc, 15 mg copper and 10 mg manganese) was procured from Wellcon Animal Health Private Limited, Agra, Uttar Pradesh, India.

Animals and experimental design

The present study was conducted in the experimental pig production farm of the Institute. Animal Ethics Committee of Institute approved the experiment protocol (approval no IAEC/L3). For this work, eight sows were randomly divided into four groups; control, multimineral (MM), multi-strain probiotic (MSP) and, MM + MSP groups. In control group, 17 piglets born from 2 sows received 1.0 mL of sterile normal saline (NSS) orally daily for seven days after birth; 22 piglets born from two sows were given 0.3 mL of MM injection in intramuscular (IM) route on days 0 and 7 of birth in group MM; 15 piglets borne from two sows in MSP group received MSP in ratio 1:1:1:1:1 at a dose rate of 10^9 CFU/piglet for first 7 days from birth, while in MM + MSP group, 23 piglets born from two sows received 10^9 CFU of MSP per piglet once daily first 7 days of birth along with 0.3 mL of MM injection IM route on day 0 and day 7. The piglets were given unlimited access to their respective sows' colostrum.

Collection of blood sample

Blood samples (1.0 mL) of piglets were collected in vial with anticoagulant (di-potassium ethylene diamine tetra acetic acid) from anterior vena cava on day 0 (prior initiation of intervention) and 7, 14 and 21 of birth.

Haematology

Haematology was performed in haematology analyser to measure haemoglobin (Hb) concentration, packed cell volume (PCV), total leukocyte count (TLC) and total erythrocyte count (TEC) in blood. Differential leukocyte count (DLC) was performed from blood smear that was stained with Giemsa stain (1:10 dilution in distilled water) manually. Total 100 cells were counted. The absolute neutrophil, lymphocyte, monocyte and eosinophil counts were derived from TLC.

Statistical analysis

The repeated measures ANOVA were applied to examine the effect of interventions on hemogram parameters. The multiple comparisons between groups, days and their interaction effects were done by using Tukey HSD test. An individual piglet served as the experimental unit. The GraphPad Prism 6 Statistics Guide was used for the data analysis. The results were presented as the mean and the standard error of the mean (SEM). Significant differences among mean values were accepted at $p < 0.05$.

Results

Hb, TEC and PCV

Statistically, a significant interaction was found between supplementations and days for TEC ($p = 0.002$) but, such interaction was not found for Hb ($p = 0.080$) and PCV ($p = 0.066$). The mean Hb concentration on day 14 ($p < 0.05$) and 21 ($p < 0.05$) and mean PCV value on day 14 ($p < 0.05$)

were significantly lower from day 0 in control group. The mean Hb concentration on day 21 in MM group was significantly ($p < 0.05$) lower as compared to day 0 value. In MSP group, the mean Hb concentration from day 7 to 21, mean PCV value on day 14 and 21 and mean TEC on day 7 were significantly ($p < 0.05$) lower as compared to day 0 value. The mean Hb concentration on day 21, mean PCV value on day 7 and 21 and mean TEC on day 7 were significantly lower ($p < 0.05$) as compared to day 0 value in MM + MSP group. When the mean Hb, PCV and TEC values were compared among the control and supplementation groups, it was noted that the mean Hb and PCV values on day 14 and mean TEC on day 7 in MM, MSP and MM + MSP groups and mean Hb on day 21 in MSP group were statistically higher ($p < 0.05$) as compared to control group (Table 1).

TLC and DLC

The mean TLC and lymphocyte count on day 7 to 21, neutrophil count on day 7 and monocyte count on day 14 were significantly ($p < 0.05$) higher from day 0 value in control group. In MM group, the mean lymphocyte count on day 14 was significantly ($p < 0.05$) higher as compared to day 0. The mean TLC and neutrophil count on day 14 to 21 and lymphocyte count on day 21 were significantly ($p < 0.05$) lower from day 0 value in MSP group. In MM + MSP group, the mean TLC on day 14 to 21 and neutrophil count on day 7 to 21 were significantly ($p < 0.05$) lower while mean lymphocyte count on day 7 and 21 was significantly ($p < 0.05$) higher from day 0 value. Statistically, the mean eosinophil count in MM, MSP and MM + MSP groups and mean monocyte count in MM, MSP and MM + MSP did not vary significantly from day 0 to 21. The average monocyte count on day 14 in control was higher ($p < 0.05$) as compared to day 0 and 7. A significant interaction was found between supplementations and days for TLC ($p < 0.001$), neutrophil ($p = 0.001$), lymphocyte ($p < 0.01$) and monocyte ($p < 0.001$) but, such interaction was not found for eosinophil count ($p = 0.396$) (Table 2).

Discussion

In this study, a significantly lower concentration of Hb on day 14 and 21 and mean PCV on day 14 from day 0 in control group might be due to either insufficient iron (Fe) reserves or rapid growth in the critical age of early life and the finding agrees with earlier report on new borne pigs (Cruz *et al.*, 2023) [2]. The insufficient translocation of Fe from sows to neonatal pigs and poor adaptation of the hematopoietic system of suckling piglets in their early life are the other attributing factors (Szudzik *et al.*, 2018) [18]. Therefore, newborn pigs frequently require additional Fe supplementation to prevent anemia (Ding *et al.*, 2020) [4]. The non-significant improvement of Hb concentration till day 14 in MM group could be due to either interference of MM with Fe absorption or unpredictable interactions among micronutrients in the body (Rossander-Hulten *et al.*, 1991) [15]. Previous research also showed that supplementation of Fe alone is more effective than MM for raising Hb levels in anemic children and non-pregnant women (Gera *et al.*, 2009) [7]. Significantly lower concentrations of Hb and PCV on day 7 to 21 in MSP group is in accordance with earlier study on suckling piglets where MSP supplementation did

not produce any substantial effect on hemogram (Hansen *et al.*, 2022) [8]. However, significantly ($p < 0.5$) higher the Hb concentration and PCV on days 14 and 21 in MSP group than control group agrees with earlier studies on grower pigs and calves fed lactic acid bacteria (LAB) including *P. acidilactici* FT28 (Dowarah *et al.*, 2018) [5]. The LAB has a positive impact on dietary non-heme Fe absorption by enterocytes through increasing productions of mucin, organic acids and microbial by-product like p-hydroxyphenyl lactic acid which facilitate the conversion of ferric iron to the more accessible ferrous form at the gut surface (Hoppe *et al.*, 2017) [10]. In our investigation, co-administrations of MM and MSP did not significantly affect the hemogram of suckling piglets, despite the fact that early life interventions with MM and probiotics are thought to have a major impact on piglet health (da Silva *et al.*, 2021) [3].

Here, the significant rise ($p < 0.05$) of TLC and lymphocyte count on day 7 to 21 and neutrophil count on day 7 in control group may be the result of the piglets' varied levels of early-life antigen exposure (Rolinec *et al.*, 2015) [14]. The activation and faster maturation of lymphocytes from the

exposure to environmental antigens also attribute to the higher counts of absolute leukocyte and its subpopulations (Siddiqui *et al.*, 2006) [17]. A transient increase of monocyte count on day 14 in control group might be a cause of temporary response to stress or exercise (Carter *et al.*, 2018) [1]. A profound increase ($p < 0.05$) of mean lymphocyte count on day 14 from day 0 in MM group could be the result of enhanced cellular immunity of piglets (Weyh *et al.*, 2022) [22]. The trace minerals augment the functioning of immune system through increased production of cytokines, proliferation of T cells including T helper cells and promoting the functions of various coenzymes (Wang *et al.*, 2025) [21]. It is worthwhile to mention here that the effect of the trace minerals on the immune system is not adequately examined in suckling piglets as compared to other farm animals. In this study, a marked decrease of mean TLC, and neutrophil count on day 14 to 21 and lymphocyte count on day 21 in both MSP and MM + MSP groups suggest that early-life supplementation of MSP either alone or in combination with MM did not produce any additional effect in modulation of leukogram of sucking piglets.

Table 1: Changes of concentration of haemoglobin (Hb, g/dL), Packed cell volume (%) and total erythrocyte count (TEC, $10^6/\mu\text{L}$) from day 0 to 21 of birth in control, multimineral (MM), multi strain probiotic (MSP) and MM + MSP groups

Parameter	Groups (n = 8)	Day 0	Day 7	Day 14	Day 21	Group mean	G	D	G×D
Haemoglobin (Hb, g/dL)	Control	10.650±0.481A,x	11.737±1.881A,x	6.550±0.882A,y	6.625±0.834A,y	8.890	0.656	<0.001	0.080
	MM	11.687±1.539A,x	10.225±1.221A,xy	8.837±0.576B,xy	7.837±0.604A,B,y	9.646			
	MSP	11.312±0.795A,x	8.662±0.332A,y	8.950±0.207B,y	8.562±0.170B,y	9.371			
	MM + MSP	9.850±0.358A,xy	10.625±0.805A,x	8.637±0.199B,yz	8.112±0.382A,B,z	9.371			
	Day mean	10.875	10.312	8.243	7.784				
Packed Cell Volume (%)	Control	36.125±1.572xy	37.762±5.022y	24.746±3.113A,z	26.517±2.630xz	31.287	0.280	0.001	0.066
	MM	38.400±4.512	33.237±3.337	33.400±1.862B	30.400±1.990	33.859			
	MSP	36.575±2.886x	29.175±0.818y	33.150±0.819B,xy	31.337±0.809y	32.559			
	MM + MSP	33.025±1.311x	29.612±0.471y	30.987±0.710B,xy	29.262±0.8180y	30.721			
	Day mean	36.031	32.446	30.570	29.379				
TEC× $10^6/\mu\text{L}$	Control	5.078±0.284A,xy	6.585±0.942A,y	4.423±0.334A,x	5.242±0.289A,xy	5.332	0.014	0.202	0.002
	MM	5.542±0.645A,x	4.695±0.438B,x	5.071±0.259A,x	5.386±0.287A,x	5.173			
	MSP	5.402±0.386A,x	4.086±0.131B,y	4.906±0.152A,x	5.423±0.204A,x	4.954			
	MM + MSP	4.607±0.167A,x	3.893±0.120B,y	4.678±0.080A,x	4.850±0.125A,x	4.507			
	Day mean	5.157	4.815	4.770	5.225				

G, group; D, day; G×D, group × day.

Means followed by different letter indicate significant (< 0.05) differences within the groups (A-C) or within the days (x-z) while share letters indicate no significant difference.

Table 2: Changes of total leucocyte count (TLC×10³/μL), differential leukocyte counts (DLC; neutrophil, N; lymphocyte, L; eosinophil, E; monocyte, M) from day 0 to 21 of birth in control, multimineral (MM), multi strain probiotic (MSP) and MM + MSP groups

Parameter	Groups (n = 8)	Day 0	Day 7	Day 14	Day 21	Group mean	G	D	G×D
TLC×10 ³ /μL	Control	6.262±0.809A,x	14.000±1.952y	13.300±1.839AB,y	14.050±1.289A,y	11.903	0.129	0.119	< 0.001
	MM	10.437±2.755AB	12.462±0.835	14.612±3.224B	9.787±0.989B	11.825			
	MSP	14.675±1.266B,x	12.925±1.494xy	9.762±0.588AB,yz	8.162±0.840B,z	11.381			
	MM + MSP	12.775±2.103B,x	10.312±0.791xy	8.187±0.760B,y	6.937±0.580B,z	6.937			
	Day mean	11.037	12.425	11.465	9.734				
Neutrophils	Control	2101.000±263.899A,x	4592.250±722.375A,y	3890.250±781.359xy	3356.250±474.312A,xy	3484.937	0.959	< 0.001	0.001
	MM	3917.375±1436.817AB	4358.000±549.453A	3619.750±987.449	1865.000±316.914B	3440.031			
	MSP	4886.625±720.994AB,x	4425.750±435.459A,x	2426.750±165.588y	1563.000±231.424B,y	3325.531			
	MM + MSP	6387.625±1750.284B,x	2745.125±217.806B,y	1871.500±233.978y	1884.750±230.201B,y	1884.750			
	Day mean	4323.156	4030.281	2952.062	2167.250				
Lymphocytes	Control	4017.875±547.133A,x	9210.500±1303.608y	8433.500±1555.280AB,y	10164.500±888.676A,y	7956.593	0.033	0.076	0.002
	MM	5787.375±1621.371AB,x	7563.250±440.052xy	10577.250±2294.604A	7457.500±747.922B,xy	7846.343			
	MSP	9054.500±954.103B,x	7893.250±1401.487xy	6725.625±587.831AB,xy	5951.750±611.565BC,y	7406.281			
	MM + MSP	5799.125±951.439AB,xy	7231.000±719.952y	5797.250±462.353B,xy	4736.625±446.430C,x	5891.000			
	Day mean	6164.718	7974.500	7883.406	7077.593				
Eosinophil	Control	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000A	0.000	0.002	0.107	0.396
	MM	107.000±79.504	0.000±0.000	0.000±0.000	0.000±0.000A	26.750			
	MSP	37.250±37.250	38.375±27.297	0.000±0.000	0.000±0.000A	18.906			
	MM + MSP	137.125±72.050	54.375±54.375	32.000±32.000	160.875±66.349B	96.093			
	Day mean	70.343	23.187	8.000	40.218				
Monocytes	Control	143.625±52.393A,x	197.250±54.7395A,x	976.250±256.724A,y	567.500±203.445A,xy	471.156	< 0.001	< 0.001	< 0.001
	MM	625.750±261.305AB	541.250±140.616B	415.500±127.290B	387.500±81.751AB	492.500			
	MSP	679.500±146.028B	432.625±93.069AB	358.375±140.578B	512.750±77.073AB	495.812			
	MM + MSP	510.750±123.831AB,x	282.000±85.587AB,xy	486.750±141.550AB,x	155.250±43.526B,y	358.687			
	Day mean								

G, group; D, day; G×D, group × day.

Means followed by different letter indicate significant (< 0.05) differences within the groups (A-B) or within the days (x-y) while share letters indicate no significant difference.

Conclusion

The findings of the current study indicate that the MSP intervention in early life effectively improves haemoglobin until weaning whereas MM augments the functioning of immune system through increased production of cytokines, proliferation of T cells including T helper cells and promoting the functions of various coenzymes. However, more research including a large number of observations on multi-location farms is necessary before the intervention can be suggested as a set of practices in swine production.

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