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Effect of feeding fermented liquid feed on haemato-biochemical parameters of pre-weaned and post-weaned young pigs of large white Yorkshire breed

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Abstract
The present study was carried out to know the effect of feeding fermented liquid feed on haemato-biochemical parameters in the pre-weaned (1st week to 4th week) and post-weaned (5th week to 10th week) young pig. Fermentation was carried out with the help of bacteria, *Lactobacillus acidophilus.* The young pigs were divided into three groups; control, treatment 1 and treatment 2. The control, treatment 1 and treatment 2 was fed on dry feed, fermented liquid feed and combination of equal parts of fermented liquid feed and dry feed, respectively, from 7 days of age to 70 days of age. Except total leucocyte count, there was significant increase in haemoglobulin, packed cell volume, total erythrocyte level in treatment 1, followed by treatment 2 and control group. The biochemical parameters like total protein, albumin and globulin were non-significantly increased in treatment 1, whereas other biochemical parameters like glucose, triglyceride and total cholesterol were either significantly or non-significantly increased in treatment 2 and control. From this study, it can be concluded that there was improvement in the haemato-biological parameters of pre-weaned and post-weaned young pigs fed on fermented liquid feed.

Keywords: Fermented liquid feed, *Lactobacillus acidobacillosis* haemato-biochemical parameters, young pigs and large white Yorkshire

Introduction
According to the 20th Livestock Census, India has 9.06 million pigs with 1.90 million Exotic/Crossbred and 7.16 million Indigenous/Non-Descript pigs. Due to stagnation in growth of poultry as well as dairy sectors, piggery sector in India have huge potential to grow in coming years. Among the other management issues, growth and survivability of young pigs during nursing and immediate post weaning period are of great concern for pig husbandry all over the world. Young pigs started to eat or drink from 2nd week of their life. That way piglet picks up the infections while licking on floor or wall of feeder/waterer through or farrowing pen. However, the level of passive immunity achieved by the piglets through ingestion of colostrum, also started to decline from 14 days of age and piglets are become more vulnerable to different infections. To minimize the difference between nutrients supply and demand of nutrients, piglets are generally offered dry creep feed from 2nd week of age. At that age, piglets enzyme system is also not fully developed and substrate of feed acts as medium for growth of different microflora in the gastrointestinal tract, leading to diarrhoea. In developed countries, good quality easily digestible feeds are available for the young pigs, which is not available in the Indian market. Due to consumption of inferior quality feed, piglets suffer from infections and require treatment containing antibiotic drugs. To minimize the infections, efforts are being made to provide probiotics to maintain the gut health of young pigs [4]. Feeding of fermented liquid milk to two weeks old piglets was found to be beneficial to reduce the incidence of diarrhoea in young pigs [3].

Weaning is a stressful events encountered by young pigs in their life, as the weaner pigs must have to rapidly adapt to changes in the diets, physical and social environments [10]. Weaning cause low feed consumption, reduced weight gain, diarrhoea, increased morbidity and mortality [14]. Weaning is mainly associated with gastrointestinal related disorders in post-weaning piglets [10], especially in early weaned pigs (weaning at 28-35 days of age). Although early weaning of piglets is beneficial to improve the sow productivity, it causes severe stress to

poorly developed gastrointestinal system. The weaning lead to reduction in the nutrient intake (energy) in post-weaned piglets [2]. The reduced energy intake associated with morphological alteration in intestine like decreased villus height and increased depth of crypt [5]. Weaning stress lead to production of excessive amount of toxin in GIT which cause destruction of villous that result in disturbance in absorption of nutrient from GIT and increased secretion of fluid and electrolyte from body and reduced enzymatic activities leading to post weaning diarrhoea [14].

To avoid infection especially during stressful event like weaning, antibiotics are commonly used along with weaner feed as preventive measures. However, due to increase drug resistance and other health related issues, use of antibiotics in livestock production is being discouraged worldwide. To counter the antibiotic resistance, alternative approaches/ products like natural growth promoters such as probiotics, prebiotics, symbiotics, enzymes, toxic binders, organic acids, oligosaccharides, phytogenic, improved feeding system etc. to improve the production in livestock sector [1].

Production of pigs without using antibiotic growth promoters represents a challenge for the pig industry. Therefore there is a need for alternatives to antibiotic which can act as growth promoter without any harmful effects on health of pigs. Fermented liquid feed (FLF) may act as potential alternative to antibiotic and other synthetic growth promoters. It has been reported that FLF increases feed utilization, digestibility and significantly improve the growth performance of pig [8, 15]. FLF provides simultaneous provision of feed and water, so there is no need to provide separate source of these nutrients [2]. Several scientific reports are available which shows that the use of fermented liquid feed (FLF) reduces the number of coli form bacteria and *Brachyspira hyodysenteriae* in the GIT of piglets and grower pigs [9]. The lactic acid producing bacteria are present in high number in the fermented liquid feed [4, 7]. Strong bactericidal action of lactic acid towards *Enterobacteriaceae* is well known [11].

Many workers in abroad have already studied the effect of FLF on performance of weaned pigs in their pig farming system. However, not much work on effect of FLF on Indian pig farming system has been done especially during pre-weaning period. Considering the above facts in mind, the present study was planned to study the effect of FLF on haemato-biochemical parameters of young pigs fed fermented liquid feed during pre and post weaning periods.

Materials and methods

Experimental design of the study

This study was carried out at Instructional Livestock Farm, Department of Livestock Production and Management, College of veterinary Sciences and Animal Husbandry, CAU, Selesih, Aizawl, Mizoram. The young pigs of large white Yorkshire breed were divided into three groups namely control, treatment 1 and treatment 2. Further each group has four replicates and each replicate has ten young pigs. A total of 120 young pigs were considered in this study. The detailed experimental design is shown in table 1.

Table 1: Experimental design of the study

S.N.	Parameters	Control				Treatment 1				Treatment 2			
1.	Number of young pigs	4 Litters				4 Litters				4 Litters			
2.	Replicates (1 litter per replicate)	CR_1	CR_2	CR_3	CR_4	T_1R_1	T_1R_2	T_1R_3	T_1R_4	T_2R_1	T_2R_2	T_2R_3	T_2R_4
3.	Type of Feed	Dry feed				FLF				Dry Feed +FLF			
4.	Feeding Period	From day 7 to day 70				From day 7 to day 70				From day 7 to day 70			
5.	Weaning Age (day)	28				28				28			
6.	Feeding Schedule	Pre-starter – 2 to 3 wks Starter – 4 to 6 wks Grower – 7 to 10 wks				Pre-starter – 2 to 3 wks Starter – 4 to 6 wks Grower – 7 to 10 wks				Pre-starter – 2 to 3 wks Starter – 4 to 6 wks Grower – 7 to 10 wks			

Preparation of fermented liquid feed

Fermented liquid feed were prepared at the pig farm of Livestock Farm Complex, College of Veterinary Sciences and Animal Husbandry as shown in fig. 1 A. Feeds for fermentation were prepared by mixing main ingredients like maize, soybean, ground nut cake and wheat bran as per required rations. Feed constituents like, mineral mixture, lysine, methionine, soybean oil and salt were added in the fermented feed just before feeding to the animals. The feed and water in the ratio of 1:1.5 was mixed to prepare the fermented liquid feed and then *Lactobacillus acidophilus* was added at concentration of 10^6 to 10^7 CFU/ ml of liquid feed [16]. For proper fermentation, liquid feed was kept under airtight condition in containers and by using room heater, required temperature of 20-25º C inside the room was tried to maintain. The FLF pH was regularly checked by using pH meter. Once desired pH of 3.5-4.5 in the FLF was achieved (Fig. 1 B), half of the FLF were used for feeding and other half were used as inoculum for fermentation of next batch of feed. For continuous production of FLF required for the experiment, *"Back slopping"* procedure as explained by Salovaara [16] was adopted.

A. B.

Fig 1: Preparation of fermented liquid feed A. Properly packed plastic container containing dry feed and water (1:1.5) and *Lactobacillus acidophilus* **B.** Fermented feed with the pH of 3.5

Management of young pigs

The young pigs were reared in farrowing pens and weaner pens which was made up of polypropylene plastic slatted floor, during pre and post-weaning periods, respectively. Farrowing pens were fitted with farrowing crate to prevent the death of piglets due to crushing. To maintain the required temperature for the young pigs the brooding facilities were provided in farrowing pens as well as in the weaner pens.

The arrangement for clean and fresh drinking water was provided round the clock through nipple drinkers. The feed was provided twice daily at 9:00 AM in the morning and at 4:00 PM in the evening and were fed up to their appetite as per NRC [12]. The amount of feed given was weighted before it was given to piglets and proper record of feeding was maintained. Pre-starter feed were provided to the young pigs during 2-3 weeks of age, starter feed during 4-6 weeks of age and then grower feed during 7-10 weeks of age. Compositions and estimated nutrients contents of different rations used in the experiment are shown in table 2.

Table 2: Composition of different rations used in the experiment

S. N.	Rations→		Pre starter Ration	Starter Ration	Grower Ration	Lactation Ration
	Age in weeks rations were fed→		**2-3**	**4-6**	**7-10**	**Lactation**
	Ingredients	Cost/kg	**Parts (%)**	**Parts (%)**	**Parts (%)**	**Parts (%)**
1.	Maize	27.97	40.55	51.55	65.90	65.90
	Soybean Meal	64.09	20	20	13	13
	Ground Nut Cake	61.62	2	2	10	10
	Wheat Bran	29.97	2	2	8	8
	Skim milk powder	350	30	20	0	0
	Soybean oil	100.00	3	2	0	0
	Methionine	700.00	0.15	0.15	0.10	0.10
	Lysine	380.00	0.3	0.3	0.2	0.2
	Mineral Mixture	140.00	1.5	1.5	1.5	1.5
	DCP	100.00	0.2	0.2	1.0	1.0
	Common salt	20.00	0.3	0.0	0.3	0.3
	Total		100	100	0.3	100
2.	Cost of ration per kg		138.54	105.62	100	39.94
3.	Nutrient Contents (*calculated*)					
	Dry Matter (%)		89.63	88.61	86.92	86.89
	Energy (DE) (Kcal/kg)		3408.49	3373.49	3322.09	3218.66
	Crude Protein (%)		22.85	20.51	18.32	16.24
	Crude Fibre (%)		2.73	2.96	4.23	4.45
	Lysine (%)		1.68	1.43	1.10	0.80
	Methionine (%)		0.67	0.58	0.43	0.35
	Calcium (%)		1.11	0.99	0.78	0.92
	Phosphorus (%)		0.74	0.66	0.57	0.68

Haemato-biochemical indices of young pigs

The haematological indices like White Blood Cells (m/mm^3), Red Blood Cells (M/mm^3), Packed Cell Volume (%), Haemoglobin (%) and Lymphocytes (%) were measured in automated blood analyser (MeletSchloesing Lab., MS4e model). Three blood samples (one ml each) from each replicate of different group were collected in EDTA vials on 28 and 70 days of age (Fig. 2). They were then immediately analysed.

On the other hand, biochemical parameters such as glucose (mg/dl), triglyceride (mg/dl), total cholesterol (mg/dl), total protein (g/dl) and albumin (g/dl) by using fully automated Dry clinical analyser (FujiFilm 4000i). Three blood samples (2 ml) from each replicate at random were collected in Clot Activator vials on 28 and 70 days of age and were allowed to be clotted for 30 minutes (Fig. 2). They were then centrifuged at 3000 rpm for 10 minutes for separation of the serum. The serum so obtained were analysed for different biochemical parameters.

Fig 2: Collection of blood from piglets through anterior vena cava

Statistical analysis

The data collected from the study were subjected to statistical analysis using IBM SPSS version-16 software for meaningful and accurate comparison and interpretation.

Results and Discussion

Haematological indices

The mean (± SE) haematological indices of LWY young pigs *viz.*, haemoglobin (Hb in g/dl), packed Cell Volume (PCV in %), total leucocyte count (TLC in m/mm^3), and total erythrocyte count (TEC in M/mm^3) on 28 and 70 days of age are presented in table 3. Statistical analysis revealed except for TLC all other haematological indices were different significant (P<0.01) among groups C, T1, and T2 on both 28 and 70 days of age. In the present study, the level of haemoglobin, packed cell volume, total leucocyte count, and total erythrocyte count were found to high in young pigs fed with FLF. Haematological parameters in treatment 1 (FLF) were found on the upper side of the normal range as shown in table 3. From these results, it can be said that FLF has a good impact on the blood parameters of young pigs as compared to pigs fed on dry feed. Not many reports are available indicating the impact of feeding the fermented liquid feed to pigs on haematological parameters. But, O' Meara *et al.* [13] reported that pigs fed fermented liquid wheat tended to have a lower percentage of lymphocytes and had a higher percentage of granulocytes than those fed fresh.

Table 3: Haematological indices of LWY young pigs under control and treatment groups (Mean±SE)

Parameters	Day	Control (C)	Treatment 1 (T_1)	Treatment 2 (T_2)	F-value	Sig.
Hb (g/dl)	28	9.41±0.07^a	10.70±0.22^b	9.68±0.15^a	18.466**	0.001**
	70	10.36±0.13^a	11.53±0.30^b	10.42±0.12^a	10.829	0.004**
PCV (%)	28	28.05±0.68^a	33.88±0.54^b	29.28±0.80^a	20.498**	0.000**
	70	32.55±0.20^a	37.88±0.54^b	33.55±0.71^a	28.989**	0.000**
TLC (m/mm3)	28	10.50±0.30	11.48±0.38	11.13±0.50	1.51^{NS}	0.272^{NS}
	70	17.20±0.55	17.08±0.74	16.78±0.51	0.129^{NS}	0.880^{NS}
TEC (m/mm3)	28	5.29±0.13^a	6.39±0.10^b	5.52±0.15^a	20.498**	0.000**
	70	6.14±0.04^a	7.15±0.10^b	6.33±0.13^a	28.989**	0.000**

**P<0.01; ^{NS} Non-significant

Note: Means bearing at least one common superscript in each row do not differ significantly

Biochemical indices

The mean (± SE) biochemical indices of LWY young pigs *viz.*, glucose (GLU in mg/dl), triglyceride (T.G in mg/dl), total cholesterol (T.CHO in mg/dl), total protein (T.P in g/dl) and albumin (ALB in g/dl) on 28 and 70 days of age are presented in table 4. Statistical analysis revealed significant (P>0.05) differences among groups C, T1, and T2 in triglyceride and total cholesterol indices on both 28 and 70 days of age, whereas in the case of globulin, the significant difference among control, treatment 1, and treatment 2 only on 28 days of age. In the present study, the level of glucose, triglyceride, and total cholesterol was high in the control group as compared to treatment 1 and treatment 2 as shown in table 4. No much literature is available showing the impact of fermented liquid feed on biochemical parameters fed to pigs. However, Wang *et al.* [17] reported that feeding fermented cotton seed meal to broiler chicken improved the levels of immunoglobulin, biochemical parameters, antioxidative abilities in serum or liver tissue, and *Lactobacilli* and total anaerobic bacteria counts in ceca digesta on days 21 and 42. Similarly, Hu *et al.* [6] reported that levels of serum total antioxidative capacity, total superoxide dismutase, total protein, albumin and glucose of birds fed fermented rapeseed meal were higher than birds fed rapeseed meal on days 21 and 42.

Table 4: Biochemical indices of LWY young pigs under control and treatment groups (Mean±SE)

Parameters	Day	Control (C)	Treatment 1 (T_1)	Treatment 2 (T_2)	F-value
GLU (mg/dl)	28	122.75±9.87	120.5±3.57	128.00±3.39	0.37^{NS}
	70	117.25±5.57	114±2.74	119.50±6.59	0.28^{NS}
T.G (mg/dl)	28	48.75±0.85^b	44.00±0.41^a	45.50±0.65^a	13.48**
	70	86.50±1.19^b	79.25±1.25^a	80.25±1.11^a	11.01**
T.CHO (mg/dl)	28	191.00±4.04^b	108.75±7.96^a	105.75±3.97^a	73.55**
	70	212.75±3.57^b	131.00±2.12^a	133.00±2.74^a	263.80**
T.P (g/dl)	28	6.03±0.18	6.1±0.18	5.88±0.10	0.54^{NS}
	70	7.75±0.29	7.95±0.28	7.28±0.09	2.13^{NS}
ALB (g/dl)	28	3.33±0.08	3.13±0.14	3.58±0.17	2.85^{NS}
	70	3.80±0.15	4.20±0.25	3.95±0.17	1.11^{NS}
GLO (g/dl)	28	2.70±0.18^{ab}	2.98±0.14^b	2.30±0.11^a	5.55*
	70	3.95±0.43	3.75±0.24	3.33±0.25	1.00^{NS}

**P<0.01; *P<0.05; ^{NS} Non-significant

Note: Means bearing at least one common superscript in each row do not differ significantly

Conclusion

All haematological indices except TLC were statistically different significant (P<0.01) among groups C, T1, and T2 on both 28 and 70 days of age. The values of all haematological parameters were high in young pigs fed with fermented liquid feed (T1), indicating the good effect of FLF on haematological indices. In case biochemical indices, significant (P>0.05) differences among groups C, T1, and T2 in triglyceride and total cholesterol indices on both 28 and 70 days of age (highest in the control group), whereas in the case

of globulin, the significant difference among control, treatment 1 and treatment 2 only on 28 days of age (highest in T1). Moreover, there was non-significant increase in total protein and albumin in treatment 1. It can be concluded from this study that the haemato-biochemical parameters of young pigs during the pre and post-weaning period were improved by fermented liquid feeding.

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