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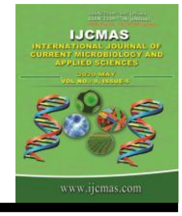

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Effect of Feeding Probiotics and Milk Powder Supplemented Creep Ration on the Blood Profile of Pre-Weaned Hampshire Piglets

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A B S T R A C T

The experiment was conducted to study the blood profile of pre-weaned Hampshire piglets fed creep ration supplemented with probiotics and milk powder. Twenty eight numbers of piglets were selected from litters of four sows, thus forming T_0, T_1, T_2 and T_3 groups having 7 piglets each and allocated to different feeding treatment. The result of average haemoglobin (Hb) (gm/100ml) (9.70±0.66, 9.85±0.65, 10.10±0.59, 11.45±0.91 in T_0, T_1, T_2 and T_3 respectively) revealed no significant difference (P <0.05) among the groups with a non-significantly higher Hb level in T_3 and T_2 when compared to T_0 and T_1. The average total protein (gm/100ml) (6.69 ±0.12, 6.92±0.18, 6.40±0.23 and 5.84 ±0.24 in T_0, T_1, T_2 and T_3 respectively) were significantly higher in T_0 and T_1 as compared to T_3. The average blood glucose (mg/100ml) (100.73±2.56, 87.27±4.33, 92.83±5.26 and 92.13±2.93) did not differ significantly (P<0.05) among the groups. However, it was slightly lower in T_1 followed by T_3 and T_2 as compared to T_0. The findings indicated that dietary supplementation of milk powder and probiotics alone or in combinations had a non-significant influence on haemoglobin, total protein and glucose levels of blood in piglets and the levels remained well within their respective normal range.

Introduction

One of the major problems in the rearing of pigs is the high mortality rate (around 20%) up to weaning age (Backstrom, 1973). Improvement in nutrition and healthcare aimed at reducing sow's stress and piglet viability is the key to successful pre-weaning piglet management. The young pigs can grow very fast but, unfortunately, suffers from several stress factor including nutritional deprivation, environment and gastrointestinal problems (Dowarah *et al.*, 2016). Due to larger litter sizes and increased competition for sow milk, nutrient availability for newly-born pigs is often limited. Though sow milk consumption remains as the main source of nutrients for neonatal pigs, providing alternative food sources may offer numerous benefits (Sulabo, 2009). The post-weaning growth of pigs is closely related to their pre-weaning health status.

A gastrointestinal infection accounts for significant financial losses in addition to animal welfare concerns (Lahteinen *et al.*, 2015). The common practice of supplementing antibiotics in livestock for improved animal performance has been condemned due to its adverse effect on animals as well as humans, the ultimate consumer of animal produce. Since then it has been the greatest challenge to farmers to rear healthy piglets devoid of antibiotics supplementation (Dowarah *et al*., 2016).

Thus the quest for finding ways to replace of antibiotics began with probiotics becoming suitable alternatives to antibiotics in piglets feeding strategies. FAO/WHO (2002) states that "Probiotics are mono or mixed cultures of live organisms which when administered in adequate amounts confer a health benefit to the host". Probiotics may contain one or more strains of microorganisms and may be given either alone or in combination with other additives in feed or water (Thomke and Elwinger, 1998).

Probiotics help establish a microenvironment in the gut that favours beneficial microorganisms and reduces the colonization of pathogenic bacteria (competitive exclusion) by: (1) creating a hostile environment for harmful bacteria species (through production of lactic acid, SCFA, and reduction in pH); (2) competing for nutrients with undesired bacteria; (3) production and secretion of antibacterial substances (e.g. bacteriocins by *Lactobacillus, Bacillus* spp.); and (4) inhibition of bacterial adherence and translocation (Nurmi and Rantala, 1973; Fuller, 1989; Netherwood *et al*., 1999; Schneitz, 2005; Ng *et al*., 2009; Brown, 2011).

The best indicator of animal's wellbeing and its potential for production is its health status (Kumar *et al*., 2012). Madubuike and Ekenyem (2006) had stated that haematology and serum biochemistry assay of livestock suggests the physiological disposition of the animals to their nutrition. Serum biochemistry is important indicator of health and disease in animals and has become indispensible in the diagnosis, treatment or prognosis of many diseases. Determination of the serum biochemistry reflects the physiological responsiveness of the animals to its internal and external environment (Esonu *et al.*, 2001). As such the study of blood profile of animals could be a scope to assess their physiological response to the nutritive ration provided to them.

Therefore, the present study was conducted to evaluate the effect of feeding probiotics and milk powder supplemented creep ration on the blood profile of piglets.

Materials and Methods

Ethical approval

The animal experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC), Assam Agricultural University, College of Veterinary science with the number IAEC 770/ac/CPCSEA/FVSc/AAU/IAEC/17-18/535 and carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA), Ministry of Environment, Forest and Climate Change, Government of India.

Experimental animals

Seven 1-week old healthy piglets of uniform size and body weight were selected each from four different litters (7x4) from sows of similar parity constituting 4 groups. Each group of the experimental piglets was randomly assigned to one of the four feeding treatments viz., T_0 (fed conventional creep feed), T_1 (fed 5% milk powder supplemented creep feed), T_2 (fed probiotics added @1g per kg supplemented creep feed) and T_3 (fed creep feed supplemented with 5% milk powder and probiotics @1g per kg supplemented creep feed).

Experimental design

All the experimental piglets were housed at the 30-sow Teaching Unit of the Department of Livestock Production and Management and they were raised entirely on their dams' milk from birth to 7 days of age. From day-8 onward, the piglets of T_0, T_1, T_2 and T_3 groups were offered respective experimental feed twice daily as per the feeding schedule up to weaning at 56 days of age. The piglets were separated from their sows daily in the morning and evening for a period of 1-2 hours following suckling and offered feed in the outdoor run and fed to appetite. The piglets were provided clean wholesome water ad libitum round the clock.

The formula of the basal conventional farm ration and also compositions of the ration, milk powder and probiotics used for the piglets are as shown in the Tables 1 and 2 respectively. The proximate analysis of the feed samples was done as per methods described in AOAC (1990).

The laboratory analyses of feed samples were done in the Department of Animal Nutrition, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, India.

Procedure for Estimation of blood parameters

Blood parameters were estimated at weaning on 56^{th} days of age of the piglets. The representative blood samples were collected from the jugular vein of the piglets using sterile syringe and needle and stored properly before examination. Laboratory analysis of blood samples were done at Department of Veterinary Physiology and T.V.C.C., College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, India.

The estimation of hemoglobin (gm/100 ml) was done from fresh blood just after collection by standard "Acid hematin method" using Sahli-Haemoglobinometer. The reading was made after the colour matches the standard of haemoglobinometer and the results were recorded in gm/100 ml blood. The Glucose (mg/100ml) and total protein (gm/100ml) estimations of the blood samples were done using commercial kits (Avantor brand).

Statistical analysis

Statistical devices like descriptive statistics, ANOVA with post hoc test (Duncan multiple range test) were used. Software package "SAS Enterprise Guide 4.3" is used to analyze the data.

Results and Discussion

The average values of haemoglobin (gm/100 ml), total protein (gm/100 ml) and glucose (mg/100ml) at weaning have been presented in Table 3.

Haemoglobin

The haemoglobin (gm/100ml) levels were observed to be lowest in T_o and tended to increase slightly in $T_{3,}$ followed by T_2 and T_1

but the difference was not significant statistically (P<0.05) among the treatment groups. Results of this research indicated that probiotics and milk powder supplementation did not show significant effect on Hb concentration of piglets. However, the slightly higher level of Hb inT_3 and T_2 groups when compared to the remaining groups (T_0 and T_1) might be an effect of probiotics and milk powder. A plausible mechanism is that probiotics increases the bioavalibility of iron (Perez-Conesa *et al*., 2007) and the effect of low pH caused by production of organic acids by probiotics. The low pH can prevent the formation of complexes with low solubility and also activate phytases. The organic acids chelate with the iron and delay the gastric emptying thus increasing the absorption of iron (Sundberg, 2011). Cetin *et al.*, (2005) found that probiotics supplementation caused statistically significant increase (P < 0.05) in the Hb values of Turkeys.

He stated in his study that dietary probiotic supplementation may prevent anaemia. The results of the current study on hematological parameter is in agreement with the findings of Rao (2007) who in his studies found no significant difference in the measured hemoglobin values between group of nursery pigs fed diets supplemented with or without *Lactobacillus*-based probiotics . Our results were also paralleled with that recorded by Dlamini *et al.,* (2017) who reported that the supplementation of probiotics did not cause significant effect (P>0.05) on Hb concentration of the piglets.

In contrast to our findings, Arab *et al.,* (2014) found that lambs receiving probiotics (Bioplus 2B) in feed had a significantly decreased (P<0.05) level of Hb. The findings of the current study also disagree with that of Mohan *et al.,* (1996) findings, who observed a significant (P<0.05) reduction in Hb content by the addition of probiotics, to a mean value of 7.9 g% compared to 9.2 g% in control birds. The researcher implied that this reduction may be caused by the competition of the probiotics with the host for folic acid or other nutrients.

Total protein

The result of the total protein (TP) was found to be significantly different though it was within the normal range of 5.8-8.3 gm/dl (Boyd, 1984) in all the treatment groups. Results showed that T_0 (basal diet) and T_1 (milk powder) had significantly higher TP as compared to T_3 (milk powder and probiotics) group of piglets. Results also showed that level of total protein was not significantly different between T_0, T_1 and T_2 groups indicating that supplementation of probiotics and milk powder separately had no significant effect on total protein when compared to control groups and this findings were in harmony with that recorded by Chen *et al.,* (2005) who concluded that there was no effect of dietary probiotics feeding on total protein of growing piglets. The present findings also revealed that TP levels of T_2 and T_3 groups are not significantly different from each other but it was slightly lower in T_3 and this may be due to the combine effect of milk powder and probiotics. These findings were similar to that reported by Pollmann *et al.,* (1980) who observed that TP level was slightly reduced with the addition of lactose in probiotics as compared to Probios feeding alone though the difference was not significant. Kumar *et al.,* (2012) observed that serum total proteins remained within normal range and did not differ significantly among different dietary treatments with or without probiotics.

The present findings were in contrast to that reported by Bera and Samanta (2005) who found that piglets with probiotics treatment had significantly higher total protein level compared to control groups.

Table.1 The formula of the basal conventional farm ration

Name of the ingredients	Parts per Hundred
Maize crush	50
Wheat bran	12
Ground nut cake(de-oiled)	27
Fish meal	8
Mineral mixture	2.5
Salt	0.5
Vitamins(A,D,E,K)	Added @200gm/100kg feed

Table.2 Composition of Farm Ration, Milk Powder (Sagar Brand) and Probiotics (Probios)

Farm ration (%)		Milk powder(Per 100gm)		Probiotics(Viable lactic acid bacteria/ gm)	
DM (basal feed)	95	Energy(Kcal)	366	*Enterococcus faecium, Lactobacillus acidophilus, Lactobacillus casei and Lactobacillus plantarum*	10 million CFU
DM(left over feed)	92	Total fat(gm)	1.5		
CP	22.1	Total carbohydrate (gm)	52		
EE	5.06	Protein(gm)	35		
CF	4.75	Sodium(mg)	549		
Total ash	7.75	Calcium(mg)	1200		
NFE	60.34				

Table.3 Average haemoglobin (gm/100ml), total protein (gm/100 ml), glucose (mg/100ml) levels of piglets of different treatment groups

Blood parameter	Groups			
	T_0	T_1	T_2	T_3
Haemoglobin (gm/100ml)	9.70±0.66	9.85±0.65	10.10±0.59	11.45±0.91
Total protein (gm/100ml)	6.69^{a} ±0.12	6.92^{a} ±0.18	6.40^{ab} ±0.23	5.84^{b} ±0.24
Glucose (mg/100ml)	100.73±2.56	87.27±4.33	92.83±5.26	92.13±2.93

(P<0.05)

*Treatment means having atleast one common superscript in a row do not differ significant

However, Arab *et al.*, (2014) reported that lambs receiving probiotics (Bioplus 2B) in feed had a significantly decreased (P<0.05) level of total protein (5.74 ± 0.3 and 5.58 ± 0.34 gm/dl with probiotics supplementation @ 0.5 and 1gm per kg feed) as compared to control group (6.27±1.0 gm/dl).

Blood glucose

In the present study, blood glucose (GLU) levels remained within the normal range of 66.4-116.1mg/dl (Boyd, 1984) in all the groups. The blood Glucose (mg/100 ml) levels of the piglets were found to be highest in T_0 followed by T_2, T_3 and T_1 groups.

However, the statistical analysis revealed no significant difference for all the treatment groups. The average mean GLU value was slightly lower with supplementation of only milk powder (T_1) followed by probiotics with milk powder (T_3) and probiotics (T_3) as compared to control group.

Similarly, Dlamini *et al.*, (2017) found no significant difference (P >0.05) in the level of blood glucose in piglets receiving diet containing probiotics when compared to the control un-supplemented group. Yadav *et al.*, (2006) also reported that rats fed with high fructose diet, the blood glucose became lower with skim milk and dahi supplementation whereas it was significantly higher in control animals and they suggested that dahi and skim milk feeding may improve insulin resistance in the skeletal muscles and adipose tissues of rats.

Arab *et al.*, (2014) reported that the levels of blood glucose was significantly decreased in lambs receiving probiotics (Glucose 57.00±12.0 and 56.31±12.0 mg/dl with Bioplus 2B @ 0.5 and 1 gm/kg feed) as compared to their control group(72.71± 8.6 mg/dl). On the contrary, Kumar *et al.*, (2012) observed an increase in the levels of serum glucose in probiotic (107.5±2.35mg/dl) treated piglet group as compared to their control group (103.8±2.13 mg/dl), though the difference was not significant. This result also did not resemble the results of Azain *et al.*, (1996) who reported that piglets from milk replacer-supplemented litters exhibited an increase in serum glucose (117mg/dl) as compared to control group (109mg/dl).

It can be concluded from present study that supplementation of probiotics alone or in combination with milk powder in diets have a slight but not a significant effect on the haematology and blood biochemistry of pre-weaned Hampshire piglets.

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