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Dietary inclusion of *Moringa oleifera* leaf powder (MOLP) improves metabolic profile of early weaned LWY piglets

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ABSTRACT

In recent years, natural feed additives are being considered as potential agents for improving metabolic health of animals by alleviating stress and increasing their digestive as well as absorptive capacity. The current study was aimed to assess the beneficial effects of dietary inclusion of *Moringa oleifera* leaf powder (MOLP) on blood metabolites of early weaned piglets. For the study, eighteen clinically healthy Large White Yorkshires (LWY) piglets weaned at 28 days were randomly grouped as Control (C), Treatment-I (T-1) and Treatment-II (T-2) with six numbers of piglets in each group. All the piglets were reared under similar management practices and routine health check-up was carried out during the study period. The control group was fed only basal feed and the T-1 and T-2 groups were fed basal feed with inclusion of MOLP @ 1% and 2%, respectively. The feeding trial was conducted for a period of 42 days. Blood sample was collected aseptically by venipuncture of anterior vencava on day 28 (i.e day of weaning), day 42, day 56 and day 70 from each piglet of all three experimental groups for estimating the major metabolic parameters. Samples were analyzed using commercially available kits in automated clinical chemistry analyzer. Findings of the study revealed that dietary inclusion of MOLP significantly improved metabolic health indices of LWY piglets during post weaning period in terms of glucose level, protein profile, lipid profile and renal function test as well as bilirubin profile. However, inclusion of 2% MOLP was more beneficial than 1% MOLP. Our study demonstrates potential health benefit of incorporating moringa leaf powder in swine feeding regimen.

Keywords: Pigs, Weaning, *Moringa oleifera*, Plasma, Metabolites.

INTRODUCTION

Antibiotic growth promoters are extensively used in livestock nutrition as stress alleviators, which results in better health and productivity of farm animals [1, 2]. It is well known fact that weaning is the most stressful event in the life of pigs. The period of suckling-weaning transition poses considerable challenges to the pig farmers for managing proper growth and health of the weaned piglets. Over the years, various growth promoters are being increasingly used in piggery to improve weight gain, feed conversion ratio (FCR) as well as to reduce morbidity and mortality of the young pigs, particularly during post weaning period [3]. Nonetheless, indiscriminate use of various growth promoters has led to antibiotic resistance, which adversely affects not only the health of the pigs but also other livestock species. Further, antibiotic resistant bacteria can also get transmitted to human being through various means and thus raise serious concern in human medicine [4,5]. Hence, it is an urgent need to search and adopt a potential alternative to antibiotic growth promoter to maintain post weaning health of piglets for profitable pig production and minimizing the issue of antibiotic resistance.

Dietary intervention is considered to be the logical approach for reducing some of the adverse effects of weaning stress [6,7]. In modern husbandry practices, natural feed additives in the form of powder or extracts are commonly used as sustainable additives with promising results [8]. The different bioactive compounds present in several plants have displayed tremendous health benefits [9]. One such important plant is *Moringa oleifera*, commonly known as 'drumstick tree', with a long history of therapeutic uses for centuries in traditional folk medicine [10,11]. Various parts of the plant including roots, seeds, flower, leaves etc. are traditionally being used as remedy for fever, diabetes, anorexia and diarrhoea [12]. However, the leaves have been extensively used and preferred due to its higher nutrient contents such as vitamins, minerals and antioxidants [13]. Phytochemicals like Astragalin, crypto-chlorogenic acid and isoquercetin are the major bioactive compounds present in moringa leaves [14, 15]. The anti-inflammatory, anti-diarrhoeal, anti-parasitic, anti-diabetic, immuno-modulatory and anti-microbial properties exhibited by moringa leaf powder are attributed to all these compounds [16]. The biological activity and therapeutic efficacy of *Moringa oleifera* leaf powder (MOLP) have been well documented by in many earlier studies [5, 7, 11, 12, 15, 17]. In view of the above, the present study was carried out to investigate the effect of dietary

inclusion of MOLP on metabolic profile of early weaned Large White Yorkshire (LWY) piglets.

MATERIAL AND METHODS

Experimental stock

The study was conducted on early weaned piglets reared in Piggery Unit, Instructional Livestock Farm Complex (ILFC), College of Veterinary Sciences and A.H., Central Agricultural University, Selesih, Aizawl, Mizoram, India.

Period of study

The study was done for a period of 6 months i.e. from April to September, 2024.

Location of the experiment

The research work of the study was carried at Department of Veterinary Physiology and Biochemistry, College of Veterinary Sciences and A.H., Central Agricultural University, Selesih, Aizawl, Mizoram, India. The city of Aizawl is located at 1020 m altitude and 23°44'12N latitude. The temperature of the study area was in the range of 20-30ºC and with a relative humidity of 45-95% during the period of research. The value mean ± SD for temperature was 23.37±2.32ºC, while that of relative humidity was 81.13±10.67 % during the study period.

Procurement and processing of *Moringa oleifera* leaves

Fresh lush green moringa leaves were handpicked from various parts of Aizawl District, Mizoram. The collected leaves were shade-dried for few days until a crispy feel to touch is obtained, while retaining its greenish coloration. Dried leaves so obtained were converted to fine powder using a household kitchen blender. Afterwards, the obtained powder was carefully packed and stored, which was later incorporated into the feed as per requirements.

Chemicals and reagents

The chemicals used in the study were of molecular biology grade and reagents were prepares with standard composition and procedure.

Ethical approval

The study was approved for experiments by the Institutional Animal Ethics Committee (IAEC) bearing approval reference number CVSC/ CAU/ IAEC/ 23-24/P-32, dt: 21/10/2024.

Experimental design and dietary treatment groups:

A total of 18 clinically healthy Large White Yorkshire (LWY) piglets were randomly selected for the study. All the selected piglets were weaned at 28 days and shifted into the weaner pens. Considering the parity, size of litter and birth weight and apparent health, all the piglets irrespective of their sex were divided into three groups *viz*., Control (C), Treatment-I (T-1), Treatment-II (T-2) comprising of 6 piglets each. Brooding facilities were provided inside the weaner pens of all the three experimental groups to maintain the required temperature for the young pigs. Routine management practices like iron injection, deworming, etc. were carried out as per standard procedure. All the piglets utilized were fed with standard rations prepared as per specification outlined by National Research Council [18] using conventional feed ingredients with the inclusion of MOLP. The piglets of the control group were fed only basal feed and those of T-1 and T-2 groups were fed basal feed with incorporation of MOLP @ 1% and 2%, respectively. The trial was carried out for a period of 42 days. Four (4) ml of blood was collected aseptically by venipuncture of anterior venacava in heparinized vacutainers from each animal of all three experimental groups at every two weeks interval during the study period i.e on the day of weaning (day 28), 14 days post weaning (day 42), 28 days post weaning (day 56) and 42 days post weaning (day 70). The collected blood samples were immediately subjected to centrifugation at 3,000 rpm for 20 minutes to separate out the plasma, which were subsequently used to evaluate the glucose and protein profile, lipid profile, renal function test and bilirubin profile using automated clinical chemistry analyzer (Fuji Dry Chem 4000i, Fujifilm, Tokyo, Japan)

Statistical analysis

Data generated in the study were analyzed by one way ANOVA using SPSS version 25.0 and the significant values in ANOVA were further tested for Tukey and Tukey'b multiple range test. Results are presented as Mean±SE. The difference was considered significant when $P<0.05$.

RESULTS

The Mean (±SE) values of glucose and protein profile is presented in Table 1. It is evident that glucose level was in the range of 101.33±0.88 to 110.00±0.57 (mg/dL) amongst the experimental groups. Its level decreased significantly ($P<0.01$) in MOLP fed piglets i.e in both the treatment groups. A declining trend of variation in glucose concentration was noticed during the study period from day 28 to day 70. The concentration of total protein was found to be varied between 4.53±0.39 to 8.21±0.13 (dL). The variation between control and treatment group was significant ($P<0.05$) on day 56, while it was highly significant on day 42 and 70. The highest level of plasma protein was found to be in treatment-II group on day 70. However, albumin level did not vary much ($P>0.05$) between control and treatment groups upto 42 days, while, a significant hike was noticed in T-2 group on 56 and 70 days. Conversely, globulin showed a significant ($P<0.05$) rise in globulin level in T-2 on 42 days, but rise in T-1 group on 56 and 70 days were highly significant ($P<0.01$). There was significantly low A/G ratio on 42 days in T-2 group and on 56 days in T-1 group.

Table 2 depicts the Mean (±SE) values of lipid profile. Observed triglycerides level ranged from 47.98 ±0.58 to 55.41±0.20 md/dL. It was found to be significantly ($P<0.01$) decreased in both the treatment groups than that of control group. However, the values in T-2 group were relatively less than those of T-1 group. The cholesterol was recorded to be in the range of 91.50±0.28 to 97.01±0.29 mg/dL. The variation observed in cholesterol level between the control and treatment groups was highly significant ($P<0.01$). Overall, there was a decline in cholesterol level in the treatment groups. Similarly, he level of HDL was found to be in the range of 55.40±0.71 to 60.16± 1.23 (mg/dL). The values of HDL increased significantly ($P<0.05$) in the treatment groups on 42 days and 70 days, but the difference was highly significant ($P=0.00$) on 56 days. The level of LDL was found to be in the range of 23.16±0.65 to 29.51 ±0.60 mg/dL. It was observed that LDL level decreased significantly ($P<0.05$) in the treatment groups on 56 days, but LDL drop was highly significant on 42 days and 70 days.

Table 3 depicts the Mean (±SE) values of renal function test and bilirubin profile of early weaned piglets. The recorded BUN value ranged from 13.56±0.56 to 20.00±2.88 mg/dL. It was found be decreased highly significantly in the treatment groups on 56 days ($P=0.000$) and 70 days ($P<0.05$). Whereas, uric acid was in the range of 0.43±0.01 to 0.62 ±0.01. It was found that uric acid level reduced significantly ($P=0.00$) in the treatment groups as compared to control group. The observed level of creatinine ranged from 0.599±0.002 to 0.611±0.001 mg/dL No significant differences were observed in creatinine level between the control and treatment groups during the study period. Similarly, the variation observed in total bilirubin level was also non-significant amongst the experimental groups although the bilirubin level ranged from 0.59±0.02 to 0.61±0.09 mg/dL during the study period.

Table 1: Glucose and protein profile of early weaned LWY piglets

Parameters	Days	Control	T-1	T-2	P-value
Glucose (mg/dL)	28	108.16±0.70	108.16±0.07	106.83±0.94	0.411^{NS}
	42	110.00^{a}±0.57	105.00^{b}± 1.15	104.33^{b}±0.88	0.001**
	56	108.66^{a}±0.80	103.16^{b}±0.30	102.00^{b}±0.89	0.003*
	70	109.33^{a}±1.45	102.00^{b}± 0.57	101.33^{b} ±0.88	0.000**
Total protein (g/dl)	28	5.71±0.42	5.73±0.41	5.78±0.31	0.217^{NS}
	42	4.53^{a}±0.39	7.13^{b}±0.18	8.15^{b}±0.16	0.008**
	56	5.91^{a}±0.41	7.68^{b}±0.67	7.12^{b}±0.33	0.031*
	70	4.81^{a}±0.26	7.6^{b}±0.15	8.21^{b}±0.13	0.001**
Albumin (g/dL)	28	3.03±0.17	3.26±0.16	3.23±0.12	0.544^{NS}
	42	2.94±0.08	3.96±0.49	3.53±0.28	0.135^{NS}
	56	3.01^{a}±0.14	3.16^{a}±0.06	4.07^{b}±0.11	0.026*
	70	3.07^{a}±0.35	3.36^{a}±0.24	4.25^{b}±0.15	0.040*
Globulin (g/dl)	28	2.88±0.005	2.41±0.01	2.55±0.03	0.644^{NS}
	42	2.31^{a}±0.02	3.11^{a}±0.008	4.31^{b}±0.01	0.013*
	56	2.53^{a}±0.01	4.31^{b}±0.07	3.02^{a}±0.003	0.004**
	70	1.50^{a}±0.01	4.26^{b}±0.008	3.03^{c}±0.005	0.000**
A/G ratio	28	1.04±0.004	1.35±0.002	1.26±0.003	0.053^{NS}
	42	1.36^{a}±0.008	1.27^{a}±0.003	0.76^{b}±0.003	0.000**
	56	1.29^{a}±0.006	0.71^{b}±0.004	1.34^{a}±0.004	0.000**
	70	2.1^{a}±0.03	0.78^{b}±0.003	1.40^{c}±0.003	0.000**

Mean± SE bearing different superscript differs significantly. (*/**) means significant and NS means non-significant.

Table 2: Lipid profile of early weaned LWY piglets

Parameters	Days	Control	T-1	T-2	P-value
Triglycerides (mg/dL)	28	55.41±0.20	54.98 ±0.17	54.34 ±0.16	0.693^{NS}
	42	55.03^{a}±0.31	51.50^{b} ± 0.28	48.20^{c} ±0.70	0.001**
	56	54.28^{a}±0.27	52.00^{b} ±0.25	47.98^{c} ±0.58	0.003**
	70	55.16^{b}±0.18	51.90^{a} ± 1.70	50.60^{a}±0.26	0.01*
Total Cholesterol (mg/dL)	28	97.01±0.29	96.58±0.15	96.70 ±0.16	0.370^{NS}
	42	96.16^{a}±0.16	93.00^{b}± 0.57	91.50^{c}±0.28	0.000**
	56	96.33^{a}±0.30	94.15^{b}±0.43	92.20^{c} ±0.30	0.004**
	70	95.00^{a}±0.001	94.00^{a} ± 0.57	92.80^{b}±0.35	0.01*
HDL (mg/dL)	28	56.33± 0.77	57.15± 0.85	57.01 ± 0.53	0.680^{NS}
	42	55.6^{b}±0.70	58.23^{a}±0.96	59.21^{a}±1.44	0.01*
	56	55.40^{c}±0.71	57.96^{b}±0.37	60.04^{a}± 0.60	0.000**
	70	56.30^{c}±0.82	58.26^{b}± 0.38	60.16^{a}± 1.23	0.04*
LDL (mg/dL)	28	29.47±0.01	29.35±1.32	29.51 ±0.60	0.09 ^{NS}
	42	28.60±0.65^{a}	26.23^{b}±3.34	24.01^{c}±2.83	0.000**
	56	29.12^{a}±1.69	28.96^{b}±2.1	27.64^{b}±2.44	0.040*
	70	29.30^{a}±0.23	28.54^{a}±0.59	23.16^{b}±0.65	0.000**

Mean± SE bearing different superscript differs significantly. (*/**) means significant and NS means non-significant.

Table 3: Renal function test and Bilirubin Profile of early weaned LWY piglets

Parameters	Days	Control	T1	T2	P-value
BUN (mg/dL)	28	19.21 ±0.26	19.34±0.27	19.43 ±0.58	0.927^{NS}
	42	20.00±2.88	17.03±0.59	15.01±1.16	0.232^{NS}
	56	19.81^{a}±0.26	16.52^{b}±0.29	14.64^{c}±0.71	0.000**
	70	18.31^{a}±0.08	15.82^{ab}±1.16	13.56^{b}±0.56	0.01*
Uric acid (mg/dL)	28	0.60±0.005	0.59±0.005	0.60±0.009	0.535^{NS}
	42	0.61^{a}±0.005	0.54^{b}±0.01	0.53^{b}±0.01	0.008**
	56	0.61^{a}±0.004	0.48^{b}±005	0.46^{b}±0.02	0.000**
	70	0.62^{a} ±0.01	0.49^{b}±0.005	0.43^{c}±0.01	0.000**
Creatinine (mg/dL)	28	0.605±0.006	0.611±0.001	0.609±0.003	0.08^{NS}
	42	0.603±0.001	0.604±0.002	0.597±0.001	0.074^{NS}
	56	0.599±0.002	0.601±0.001	0.600±0.001	0.834^{NS}
	70	0.603±0.001	0.600±0.009	0.599±0.002	0.161^{NS}
Total bilirubin (mg/dL)	28	0.61±0.09	0.61±0.02	0.61±0.07	0.912^{NS}
	42	0.60±0.05	0.61±0.03	0.60±0.08	0.845^{NS}
	56	0.60±0.15	0.59±0.08	0.60±0.10	0.761^{NS}
	70	0.59± 0.05	0.60±0.15	0.59±0.02	0.684^{NS}
Direct bilirubin (mg/dL)	28	0.31±0.09	0.30±0.02	0.30±0.07	0.601^{NS}
	42	0.29±0.05	0.31±0.03	0.30±0.08	0.592^{NS}
	56	0.29±0.15	0.29±0.08	0.30±0.10	0.891^{NS}
	70	0.30± 0.05	0.30±0.15	0.29±0.02	0.432^{NS}
Indirect bilirubin (mg/dL)	28	0.30±0.09	0.31±0.02	0.31±0.07	0.532^{NS}
	42	0.31±0.05	0.30±0.03	0.30±0.08	0.659^{NS}
	56	0.31±0.15	0.30±0.08	0.30±0.10	0.732^{NS}
	70	0.29± 0.05	0.30±0.15	0.30±0.02	0.754^{NS}

Mean± SE bearing different superscript differs significantly. (*/**) means significant and NS means non-significant.

DISCUSSION

Present study revealed that glucose level was significantly lower in treatment groups as compared to control group, which corroborates with earlier studies in other animal species [19,20]. Conversely, as study by Wankhede *et al.* [21] found elevated level of glucose in goats and ewes fed ration incorporated with MOLP. Alwaleed *et al.* [22] also observed higher blood glucose in broiler chicks fed MOLP supplemented diets. The lower level of glucose found in our study may be attributed to the abundance of flavonoids in moringa leaves. A reasonable explanation for the findings of this study may be linked with the report of Gupta *et al.* [23], who demonstrated that bioflavonoids content of moringa leaves, are responsible for stimulating uptake of glucose in peripheral tissues. Furthermore, they activate glucose-induced secretion of hypoglycemic hormone like insulin from the beta-cells of pancreas or stimulate glycogenesis, thus leading to decrease blood glucose levels.

A significant increase in the total protein concentration was found in the treatment groups than that of control group. Albumin concentration was numerically higher in T-2 group, but the hike was significant on 56 days and 70 days in T-2 group. Similarly, globulin concentration was also higher in T-2 group although the differences were highly significant on 56 day and 70 days. Our findings are supported by report of Afzal *et al.* [24] in case of goats. A study by El-Badawi *et al.* [25] also revealed that, globulin increased considerably ($P < 0.05$) in the lactating buffaloes fed with diets with inclusion of moringa leaf powder. A/G ratio is the index of general health and nutritional status of animals. In our study there was no definite pattern of variation in A/G ratio amongst the control and treatment groups. However, there was significant drop 42 days in T-2 group and on 56 days in T-1 group. Nonetheless, the values were within the physiological range reported by Dhanotiya [26]. *Moringa oleifera* leaves are superb source of proteins and have favorable composition of amino acids required for animal nutrition. The phytochemicals present in the leaves are also been reported to improve microbial protein synthesis in the gut of the animals [27]. The increased in total protein and albumin concentration observed in our study may be ascribed to higher nitrogen retention by the piglets supplemented with MLOP. The obtained levels of total protein, albumin and globulin in the current study indicate nutritional adequacy of the dietary amino acids and proteins in the piglets fed with MLOP supplemented feed.

Lipid profile indicated that plasma triglycerides were significantly lower ($P<0.01$) in the treatment groups. Our result corroborates with the findings of previous studies by Abu-Hafsa *et al.* [28], who observed that the levels of triglycerides in serum was lowered significantly in broiler chicks upon incorporation of *M. oleifera* leaf powder in their diet. The reduction in triglycerides may be due to hypo-lipidemic effects as documented by Akib *et al.* [29]. There was significant decrease in plasma total cholesterol in the treatment groups compared to control during the study period. The drop in level of cholesterol in both the treatment group is in same line with the previous studies Mahmoud *et al.* [30], who found reduced level of cholesterol in broilers fed with 3% moringa leaf meal. Our findings corroborate with those of earlier reports [21,31]. The significant decrease in serum cholesterol level observed in the study could be resulted from functional effects associated with saponin and tannins present moringa leaf. Saponin and tannins form and insoluble complexes binding to cholesterol and thus hinder its absorption [32]. Further, Jain *et al.* [33] documented that flavonoids of moringa leaf decreases HMG-CoA reductase activity and thereby inhibit biosynthesis of cholesterol. The HDL level was found to be significantly increased in both the groups; while, LDL

level decreased in the treatment groups. Results of the current study are at par with that of Mousa *et al*. [34]. Previous study by Mehta *et al*. [35] demonstrated that moringa leaves exhibit a hypo-lipidaemic effect and thus led to reduction in LDL level in rabbits. The raised level of HDL in the treatment groups is associated with increased activity of hepatic lipase [36]. Similarly, Halaby *et al.* [37] reported that moringa leaves also possess a bioactive compound 'β-sitosterol' with exhibited bad cholesterol lowering effects, which could be responsible for lower level of plasma LDL observed in this study.

The BUN level was found to be less in the treatment groups, but the reduction in BUN level was significant on 56 and 70 days. It is similar with the findings of Aljohani and Abduljawad [20], who reported supplementation dried moringa leaves in rabbits led to considerable drop in blood urea nitrogen. Previous study on goats by Khalel et al. [38] also supports our results. However, this findings in not compatible with the findings of Biswal *et al.* [39], who recorded that that the treatment group had increased level of blood urea nitrogen level than that of control group when the goats were fed with moringa leaf meal. BUN is an indicator of degradation of protein. The lower BUN observed in this study may be attributed to a balanced-energy protein ration and decreased catabolism of protein and increased availability of biologically important amino acid residues in the moringa leaf powder [40]. Similarly, uric acid was found to be dropped significantly (P=0.00) in the treatment groups when compared with control group. Our findings are at par with Tijani *et al*. [41] who recorded that there was significant decrease (P<0.05) in the level of uric acid in the birds fed with 20% *Moringa oleifera* leaf meal. Uric acid is a product of the purine metabolism and is a useful biomarker for nutritional health of animals. The decreased level of uric acid found in our study may be due to inhibition of xanthine oxidase by saponin of moringa leaves [42]. Nonetheless, plasma creatinine level is not varied significantly between treatment and control groups, although he data numerically varied. Similar results were also obtained by Al-Juhaimi *et al*. [31] in goats. On the contrary to our finding, Jiwuba *et al*. [43] stated that when African dwarf goats were fed moringa leaf, the creatinine concentration were noted to be substantially higher (P<0.05) in the treatment groups. Creatinine is a by-product of muscle metabolism, and its plasma concentration is directly associated with muscle mass. Therefore, the numerical discrepancies found in creatinine concentration during the study period may be attributed to individual variation of the piglets. There was also apparent variation in the level of total bilirubin, direct bilirubin, and indirect bilirubin. However, the variations were statistically not significant and were within the physiologically normal range. Based on our result, it can be stated that feeding of moringa leaf powder has no effect on total bilirubin, direct bilirubin and indirect bilirubin in the piglets.

CONCLUSION

It may be concluded that inclusion of both 1% and 2% MOLP in piglets' feed resulted in considerable improvement of health of early weaned Large White Yorkshire piglets in terms of metabolic profile. Nonetheless, inclusion of 2% MOLP was found to be more effective since it led to better health indices as compared to 1% MOLP. Our findings suggest that MOLP is a promising alternative candidate for development of feed additive in order to improve post weaning health of early weaned piglets. However, a comprehensive study is warranted with different level of MOLP inclusion and a large sample size for validating the findings of the present study as well as to fully explore therapeutic potential of *M. oleifer*a leaves.

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Conflict of interest

The authors declared no conflict of interest.

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