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Effect of Feeding Moringa Leaf Powder on Antioxidant Status of Early Weaned Large White Yorkshire Piglets Reared under Agro-climatic Condition of Mizoram, India

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ABSTRACT

The experiment was conducted during April to September, 2024 at C.V.Sc and A.H., CAU, Aizawl, Mizoram, India to determine the effect of feeding Moringa leaf powder (MLP) on antioxidant status of early weaned Large White Yorkshire piglets. For the study, a total of 18 clinically healthy piglets weaned at 28 days were categorized as Control (C), Treatment-I (T_1) and Treatment-II (T_2) with 6 (six) piglets in each group. The control group was fed basal feed and T_1 and T_2 groups were fed basal feed with incorporation of MLP @ 1% and 2%, respectively. The trial was carried out for a period of 42 days. Blood was collected on day 28 (i.e day of weaning), day 42, day 56 and day 70 from each piglet of all groups for estimating the major antioxidant parameters. The antioxidants estimated were superoxide dismutase (SOD), catalase (CAT), malanaldehyde (MDA) and glutathione peroxidase (GPx). The activities of SOD varied from 0.75±0.013 to 2.01±0.005 IU ml^{-1} amongst the experimental groups, whereas activities of CAT ranged from 1.48±0.02 to 2.32±0.012 IU ml^{-1}. The GPx activities were in the range of 231.93±0.49 to 234.01±0.018 (pg ml^{-1}) and MDA concentration was in the range of 218.47±0.015 to 254.31±0.013 (ng ml^{-1}). The study established that dietary inclusion of Moringa leaf powder improved the antioxidant arsenals of the weaned piglets, which in turn led to reduced weaning stress and resulted in their better growth. Further, data obtained in the study pertaining to SOD, CAT, GPx and MDA may be used as reference values for any future investigation.

KEYWORDS: Weaning, piglets, moringa, plasma, antioxidant

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

India is endowed with vast diversity of livestock wealth and 25% of the agricultural GDP is contributed by the livestock sector. Among different livestock species, pig is one of the major contributors to India's GDP (Thomas et al., 2021). As per 20th Livestock census, India has 9.06 million pigs and accounts about 1.7% of the total livestock population of the country (Mathew et al*.,* 2021). Unlike other species, pig has a great potential to contribute to faster economic return to the farmers due to certain inherent traits such as better-feed conversion efficiency, high fecundity, early maturity and short generation interval. It has immense prospective to ensure nutritional and economic security for the poor. In the North-eastern region of India, pig is traditionally being raised by economically weaker section of the society. It also substantially contributes to the economy of the tribal communities (Tochhawng and Rewani, 2013). In last decade, piggery has emerged not only as one of the major source of incomed for tribal community, but also facilitated significantly in bridging the gap between demand and supply of protein (Adesehinwa et al., 2024). From its humble beginnings in backyard setups, pig farming has evolved over the years to become a flourishing industry putting a giant step towards becoming one of the most profitable livestock enterprises of our country. Among the North-eastern states, pig is socio-culturally important species in the state of Mizoram. It is almost raised by every Mizo families as large scale farm or in their backward (Vanlalmalsawma and Sharma, 2015). Despite the promising scope and commercial importance, pig farmers face many challenges. One such challenge is health management, particularly alleviating post weaning stress (Su et al., 2022). Over the years, many strategies have been adopted to reduce post weaning stress, but with a little success. Dietary intervention is considered to be the logical approach to improve intestinal health for minimizing weaning stress (Tang et al., 2021). Accordingly, attempts are being made to modulate the gut microflora to exert beneficial effects on the host by using phytobiotics i.e the use of medicinal herbs or plants. One such plant is *Moringa oleifera*, commonly called as 'Moringa' (Pareek et al., 2023). The biological activity and medicinal properties of *Moringa oleifera* leaf have been well documented by various *in vitro* assays in previous studies (Kikusato, 2021). The richness of the leaves in various phyto-chemicals such as phenolic acids, flavonoids and carotenoids makes them potential therapeutic agents (Kashyap et al., 2022). Astragalin, crypto-chlorogenic acid and isoquercetin are the major active compounds present in moringa leaf (Salem et al., 2020). The anti-inflammatory, anti-parasitic, anti-diabetic, anti-oxidant, immune-modulatory and anti-microbial properties exhibited by moringa leaf powder are attributed to all these compounds (El-Sayed and Fathy, 2019). Feeding of Moringa leaves have also been reported to improve the anti-oxidant status and reproductive performance in mice and sows (Sun et al., 2020, Zheng et al., 2023,). Its high phenolic content is predominantly responsible for its antioxidant effects. Nonetheless, no study is carried out till now to evaluate the beneficial effects of *Moringa oleifera* leaf powder on health of early weaned piglets. Accordingly, present study attempted to assess the effect of dietary supplementation Moringa leaf powder on vital antioxidant parameters of early weaned piglets.

2. MATERIALS AND METHODS

The experiment was conducted for a period of six months during April to September, 2024 at C.V.Sc and A.H., CAU, Aizawl, Mizoram, India. A total of 18 clinically healthy Large White Yorkshire (LWY) piglets reared in the Livestock Farm, College of Veterinary Sciences & Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India was 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 Anonymus (2012) specification by using conventional feed ingredients with the inclusion of Moringa leaf powder (MLP). The piglets of the control group were fed the basal feed and those of T_1 and T_2 groups were fed the basal feed with incorporation of MLP @ 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 vena cava in heparinized vacutainers from each animal of experimental groups at 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 mins to separate out the plasma, which were subsequently used to determine the level of important antioxidants following the principle sandwich ELISA using by commercially available kits (ELK Biotechnology CO., LTD., Denver, USA).

Data obtained in the study was analysed using one way ANOVA and the significant values in ANOVA were further tested for Tukey and Tukey'b multiple range test. The data

obtained were analysed using SPSS version 25.0. Results are presented as Mean±SE. The difference were considered significant when $p<0.05$

3. RESULTS AND DISCUSSION

Table 1 depicts the SOD activities in the early weaned Large White Yorkshire piglets of control and treatment groups recorded during present study.

Table 1: Mean±SE of SOD (IU ml^{-1}) of weaned LWY piglets

Age (Days)	Control (C)	Treatment-I (T_1)	Treatment-II (T_2)	p value
28	0.82± 0.19	0.75± 0.013	0.82± 0.011	0.357^{NS}
42	0.96^{c}± 0.0.008	1.56^{b}± 0.01	1.49^{a}± 0.015	0.000**
56	1.07^{c}± 0.009	1.74^{b}± 0.015	1.87^{a}± 0.008	0.002**
70	0.95^{c}± 0.01	1.91^{b}± 0.01	2.01^{a}± 0.005	0.000**

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

The levels of SOD for control group were 0.82±0.19, 0.96±0.0.008, 1.07±0.009, 0.95±0.01 IU ml^{-1} on 28 days, 42 days, 56 day and 72 days, respectively While for the treatment-I group, the levels were 0.75±0.013, 1.56±0.01, 1.74±0.015, 1.91±0.01 IU ml^{-1} on 28 days, 42 days, 56 days and 72 days, respectively. For the treatment-II group, those were 0.82±0.011, 1.49±0.015, 1.87± 0.008, 2.01±0.005 IU mL^{-1} on 28 days, 42 days, 56 days and 72 days, respectively. It was observe that there was significant (p=0.00) hike in the activity of SOD in the treatment groups as compared to control group. It is in accordance with the report of Abu-Hafsa et al. (2020) where dietary supplementation of *M. oleifera* leaf was found to increase SOD activities. Further, Yang et al. (2020) has reported the similar pattern of variation in SOD activities in case laying ducks fed with diets containing Moringa stem meal. Likewise, Shen et al. (2021) also reported that serum SOD activities got elevated significantly ($p<0.05$) in laying chickens fed MLP (2.5%) as compared to that of control group. On the contrary, Rao et al. (2018) observed no significant difference in SOD level in broiler chicks fed Moringa leaf meal supplemented diet.

Table 2 depicts the CAT activities in the early weaned Large White Yorkshire piglets of control and treatment groups recorded during present study.

The observed CAT activity for control group were 1.59±0.013, 1.49±0.014, 1.48±0.02, 1.48±0.02, 1.52±0.01 on 28 days, 42 days, 56 days and 72 days, respectively. While, for the treatment-I group, the values were 1.70±0.016,

Table 2: Mean±SE of CAT (IU ml^{-1}) of weaned LWY piglets

Age (Days)	Control (C)	Treatment-I (T_1)	Treatment-II (T_2)	p value
28	1.59± 0.013	1.70± 0.016	1.62± 0.008	0.06^{NS}
42	1.49^{a} ±0.014	1.94^{b}± 0.0.17	1.86^{c}± 0.011	0.002**
56	1.48^{a}± 0.02	1.96^{b}± 0.010	2.07^{b}± 0.008	0.000**
70	1.52^{a}± 0.01	2.13^{b}± 0.009	2.32^{c}± 0.012	0.004**

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

1.94±0.0.17, 1.96±0.010, 2.13±0.009 on 28 days, 42 days, 56 days and 72 days, respectively. And for treatment -II group, the values were 1.62±0.008, 1.86±0.011, 2.07 ±0.008, 2.32±0.012 on 28 days, 42 days, 56 days and 72 days, respectively. The catalase activity was found to be significantly high (p=0.00) in both the treatment groups than those of control group. Our result is in agreement with that of Juhaimi et al. (2019) wherein the catalase activities increased significantly in goats fed with ration containing Moringa leaf powder. However, Rao et al. (2018) observed that catalase activities did not differ significantly between the group of broilers fed basal diet and the group fed *M.oleifera* leaf meal. Conversely, the CAT activity was found to be marginally reduced in the treatment group than the control group by Sun et al. (2020).

Table 3 depicts the level of MDA in the early weaned Large White Yorkshire piglets of control and treatment groups recorded during present study.

The MDA values observed for control group were 254.12±0.008, 255.04±0.009, 253.13 ±0.011, 238.94±0.008 on 28 days, 42 days, 56 days and 72 days, respectively.

Table 3: Mean±SE of MDA (ng ml^{-1}) of weaned LWY piglets

Age (Days)	Control (C)	Treatment-I (T_1)	Treatment-II (T_2)	p value
28	254.12± 0.008	253.98± 0.005	254.31± 0.013	0.056
42	255.04^{a}± 0.009	234.31^{c}± 0.011	242.54^{b}± 0.016	0.007**
56	253.13^{a}± 0.011	228.49^{c}± 0.006	233.06^{b}± 0.017	0.004**
70	238.94^{a}± 0.008	229.09^{b}± 0.012	218.47± 0.015	0.000**

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

Whereas for the treatment-I group, the values were 253.98±0.005, 234.31±0.011, 228.49±0.006, 229.09±0.012 on 28 days, 42 days, 56 days and 72 days, respectively. For the treatment-II group, the values were 254.31±0.013, 242.54±0.016, 233.06±0.017, 218.47±0.015 on 28 days, 42 days, 56 days and 72 days, respectively. The MDA value was significantly less (p=0.00) in the treatment groups as compared to control group. This finding is at par with that of Khan et al. (2022), who observed a drop in MDA concentration in broilers supplemented with Moringa leaf extract in drinking water. Similarly, Babiker et al. (2016) stated that sheep and goat fed with Moringa leaf had substantially less MDA in their serum. Further, Salem et al. (2020) recorded that the rabbits fed with 20% Moringa leaf had the lowest MDA, followed by level 30% and level 10%, respectively. In contradiction to our study, Shen et al. (2021) observed no difference in serum MDA levels between the experimental groups of laying chickens.

Table 4 depicts GPx activities in the early weaned Large White Yorkshire piglets of control and treatment groups recorded during present study

Table 4: Mean±SE of GPx (pg ml^{-1}) of weaned LWY piglets

Age (Days)	Control (C)	Treatment-I (T_1)	Treatment-II (T_2)	p value
28	234.06± 0.01	233.98± 0.14	233.11± 0.017	0.054^{NS}
42	232.89± 0.17	234.01± 0.018	233.73± 0.009	0.062^{NS}
56	231.93± 0.49	232.43± 0.011	232.84± 0.009	0.056^{NS}
70	233.48± 0.016	233.10± 0.018	232.79± 0.023	0.075^{NS}

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

The GPx level for control group were 234.06±0.01, 232.89±0.17, 231.93±0.49, 233.48±0.016 on 28 days, 42 days, 56 days and 72 days, respectively. For the treatment-I group, the levels were 233.98±0.14, 234.01±0.018, 232.43±0.011, 233.10±0.018 on 28 days, 42 days, 56 days and 72 days, respectively. While for the treatment-II group, the GPx levels were found to be 233.11±0.017, 233.73±0.009, 232.84±0.009, and 232.79±0.023 on 28 days, 42 days, 56 days and 72 days respectively. The GPX values were found to be varied apparently, but the variations were not-significant (p>0.05). This is in accordance with Kekana et al. (2020), who also reported a no significant variation results pertaining to GPX activities in Moringa fed crossbred cattle. On the other hand, Wafa et al. (2017) found that GP_X activity increased when cattle are fed with ration containing 4% and 8% Moringa leaves. Mousa et al. (2017) reported that Japanese quail that received feed additives containing 0.4% Moring a leaf meal during the laying phase had a significantly higher level of GPx activities.

Oxidative stress is inevitable in case of weaning piglets (Yu et al., 2021). Reactive oxygen species (ROS) such as H_2O_2 and superoxide are constantly generated from oxygen in all aerobic metabolisms. However, the process of weaning lead to an imbalance between the production of ROS and their neutralization by antioxidant machineries of the body. The imbalance so created cause oxidative stress that damages intestinal epithelium resulting in inflammatory condition and diarrhoea (Zheng et al., 2017). Previous studies demonstrated that the ROS induce tissue and cell damage by apoptosis (Song et al., 2016; Li et al., 2020). Weaning also alters the immune mechanism of the piglets making them more susceptible to various pathogenic microbes (Cao et al., 2018). Antioxidants play vital role in preventing or slowing down the oxidative damage (Miller et al., 1993). Amongst the different type of antioxidants, enzymatic antioxidants viz. SOD, CAT, GPx etc are the most predominant and plays active part in minimizing oxidative stress (Celi et al., 2010). Yin et al. (2014) observed that the activities of SOD, CAT are inhibited by early weaning of piglets and thus make them more prone to stress. Therefore, maintaining post weaning health of the piglets is essential for profitable pig farming.

Our study confirmed that Moringa leaves hold tremendous potential in improving the antioxidant system of piglets as indicated by higher level of SOD, CAT and lower level of MDA in the treatment groups fed diet with inclusion of Moringa leaf powder. The increase in activities of SOD and catalase observed in our study may be attributed to high levels of antioxidants such as glutathione, flavonoids, ascorbic acid and beta carotene present in Moringa leaves. These antioxidants help the body to defend against stress by up-regulating the expression of antioxidant enzymes (Mensah et al., 2012). The lower level of MDA activity found in both the treatment groups may be due to the presence of high phenolic contents *M. oleifera* leaves (Bamishaiye et al., 2011).

4. CONCLUSION

Feeding of Moringa leaf powder to early weaned piglets improved their antioxidant profile, which in turn resulted in reduced oxidative stress during the post weaning period. It may be concluded that Moringa leaf is a promising supplementary feed source to improve post weaning health of piglets in terms of antioxidant enzymes.

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